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**Studies on the Effects of Salinity and Heavy Metals
on the Sporophytic & Gametophytic generations of
Arabidopsis thaliana (L.) Heynh.**

by

ALI HASSAN RIND BALOCH

MSc. (Agri.) Hons. (Pakistan)

*A thesis submitted in candidacy for
the degree of
Doctor of Philosophy*

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**Department of Biological Sciences
University of Durham
England (U.K.)
April, 1994**



27 JUL 1994

**This thesis is entirely my own work &
has not previously been
offered in candidature
for any other degree
or diploma.**

**Ali Hassan Rind Baloch
April, 1994**

DEDICATED WITH LOVE

TO

**My parents (Mohammad Moosa, Bhanwari), my wife (Shazia Hassan)
& my children (Wazeran, Ismail, Waheed, Zareena, Rubeena, Sattar, Hamid,
Majeed, Murtza, Rabia) whose longings kept me working from Dawn to Dusk.**

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ABSTRACT

BY
ALI HASSAN RIND BALOCH

The aim of this research was to study the effects of Salinity & Heavy Metals on the Sporophyte & Gametophyte generations of *Arabidopsis thaliana*.

After developing a pollen germinating protocol, some basic experiments were conducted to determine the effects on chemical & physical parameters on pollen germination & pollen tube growth. Additions of heavy metals & high concentrations of buffer (Tris-HCl) in pollen germinating medium (PGM), inhibited pollen germination (PG) & pollen tube length (PTL), while pH range (7.0-9.0) had little effect. The idea of 2-fold effect (nutritional & osmotic) of sucrose was supported in results. *In vivo* pollen growth was much greater than *in vitro* & genotypic differences occurred among the mutants for PG & PTL. Comparing the pollen nutritional requirements for PG & PTL between *Cicer arietinum* & *A. thaliana*, it was noted that the pollen of the former had a greater requirement for H_3BO_3 & $Ca(NO_3)_2$ than latter.

The range of temperatures (0°C-35° C) studied, indicated that the most favourable temperature for PG was 20°C & for PTL 25°C, while extreme temperatures (0°C, 35°C) were harmful to *A. thaliana* pollen. 20°C was found to be optimum temperature for PG & PTL of *C. arietinum*.

Correlation between pollen tube growth rates (PTGRs) and sporophytic traits of F1s showed a generally strong positive correlation with most of sporophytic traits, but a very loose to negative correlation for earliness parameters.

Recording the impacts of salinity, parallel effects were found for the sporophytic & gametophytic generations of the plant, as reported by earlier workers. The results also indicated that it is possible to develop a pollen plant salinity index (PPSI) for plant species under specific plant growing conditions. It was also observed that salinity stress during pollen gametogenesis preconditioned pollen to high salinity levels, resulting in higher PG & PTL than in pollen raised under non-saline conditions. Similarly, the fruit-setting, seed-setting & seed-filling were relatively less affected by saline-stress, in progeny when pollen and pistillate plants were grown under similar saline regimes. Anatomical studies showed that salinity induced changes in the epidermis, cortex, pericycle, xylem, pith & cross sectional diameter of vascular bundle (CSDVB) in stem, leaf & root tissues.

Lower reductions for the fruit-setting, seed-setting & seed-filling were recorded in response to the high heavy metal concentrations in progeny when pollen and pistillate plants were raised under similar heavy metal regimes. It was apparent that pollen become conditioned to a stress environment during its development in the anthers of stressed plants, & this preconditioning allowed its pollen tubes to grow more successfully in the styles of the female parents growing in a similarly stressed environment, where the pistil may have accumulated higher levels of heavy metals.

Heavy metals induced changes in stem shape, epidermis, cortex, vascular bundles & chloroplasts in stems. The metals induced changes in the leaf thickness, epidermis, palisade cell size, spongy mesophyll area, size of intercellular spaces & chloroplasts in the leaf, causing disruption of cortical layers, lignification of pericycle & phloem, damage to the endodermis and increase in xylem cell size & stele diameter in roots.

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ABBREVIATIONS

- ABA = Absciscic acid B/P = Branches per plant
BV = Blueviolet
CSDVB = Cross section diameter of vascular bundle
DAP = Days after planting
DTB = Days to bolting
FAA = Formaldahyde/acetic acid/alcohol fixative
FCR = Fluorochromatic reaction
FDA = Fluorescein diacetate
FFI = First flower initiation
Fig. = Figure
Figs. = Figures
HS = High-stress
LM = Light microscopy
M = Molar
MS = Male sterile
MSI = Main shoot/raceme initiation
MSS = Murashige & Skoog solution
PDs = Pollen donors
PDW = Plant dry weight
PG = Pollen germination
PH = Plant height
PLL = Pollen lethal level
PPSI = Pollen plant salinity index

P/P = Pods per plant
PTGRs = Pollen tube growth rate
PTL = Pollen tube length
RD = Rosette diameter
RDW = Root dry weight
SD = Standard deviations
SDW = Shoot dry weight
SF = Stress-free
SM = Standard medium
SS = Salt stressed
TB = Toluidine blue
Temp. = Temperature
TS = Transverse section
UV = Ultraviolet
V = Violet
WT = Wild type
cm = Centimeter
d = Days
g = Gram
h = Hour
hrs = Hours
l = Litre
min = Minutes
ml = Millilitre
mM = Millimolar
mm = Millimeter

ndv = Non-determined value

ns = Non-significant

ppm = Parts per million

w/v = Weight/volume

% = Percent

μm = Micrometer

$^{\circ}\text{C}$ = Degree celsius

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Chapter I

INTRODUCTION

The fruit fly, *Drosophila*, has been a model organism for geneticists for many years and its importance in genetics research is widely acknowledged. Recent developments in plant molecular biology mean that for many biologists around the world, an apparently insignificant weed, *Arabidopsis thaliana*, is poised to become a “Botanical *Drosophila*” (Zoe et al., 1991).

1.1 Research importance and distribution of *Arabidopsis*

Arabidopsis thaliana has been known by many colloquial names like “mouse-ear cress”, “wall cress” and “thale cress”. The latter is currently in common usage (Zoe et al. 1991).

Though many plant species are very important for economic reasons, breeders and geneticists have also customarily used many plants for genetic exploitation and experimentation in applied research. But because of their long generation time and space requirements, most crop species cannot be considered ideal for genetic research. In contrast *Arabidopsis thaliana*, a small flowering weed plant with unique qualities is considered as excellent model for the genetic studies. Its use for classical genetics goes back over 45 years and it has recently been adopted by number of laboratories for use in molecular genetic approaches to problems in plant physiology, biochemistry and development (Elliot, 1989). Its short life cycle



and small size makes it more valuable for screening mutagenetic agents. Because of its extremely small nuclear volume, *Arabidopsis* appears to be the most radiation resistant among the higher plants (Sparrow et al. 1973). A substantial base of genetic information already exists and a diverse collection of mutants have been isolated and used to address a number of questions in plant biochemistry and physiology (Estelle and Somerville, 1986).

1.1.1 Ecological distribution

Arabidopsis and its allied relatives are widely spread on the earth, especially in the northern hemisphere (Redei, 1970). The gene centre is probably in western or north-western Europe. The most conspicuous variation in the species is in the response of flowering to natural day length and temperature regimes (Laibach, 1951; Jones, 1971; Harer, 1951; Napp-zinn, 1962, 1963, 1964; Ratcliff, 1961).

1.1.2 Special features

Laibach (1907), found that in the interphase nucleus the number of heterochromatic bodies were identical with the number of chromosomes seen at metaphase, an uncommon phenomenon in the majority of plants. Laibach (1943) was also first to point out some of features of *Arabidopsis* that suit it for laboratory work in classical genetics.

Some advantages in *Arabidopsis* plants for genetic manipulations listed by Redei (1975) include:

(a) The diploid chromosome number is five pairs (Laibach, 1907; Steinitz-Sears, 1963) Autotetraploids are cytologically stable and genetically normal (Bouharmont, 1969; Redei, 1964).

(b) All primary trisomics are morphologically distinguishable (Steinitz-Sears, 1963; Lee-cheen and Steinitz-sears, 1967).

(c) The life cycle may be completed within one month or it may be extended to several, depending on photoperiodic exposure (Laibach, 1951; Redei, 1962).

(d) In the laboratory, outcrossing is maximally in the 10^{-4} range (Redei unpublished) and in nature it does not exceed 2% (Snape and Lawrence, 1971; Jones 1971).

(e) The seed output of a plant may exceed 50,000.

(f) For phenotypic classification 5-10 or more plants may be crowded in 1cm^2 area (Myerscough and Marshall, 1973) making it possible to study very large populations.

(g) Plants can be grown to maturity on simple, well-defined media in standard size aseptic test tubes (Laibach, 1943; Langridge, 1957 and Redei, 1965), liquid culture is practical (Redei, 1974) and the callus grows satisfactorily in both diploid (Ziebur, 1965; Yokoyama and Jones 1965; Shen-Miller and Sharp, 1966; Anand, 1966) or haploid states and can be redifferentiated into plantlets (Gresshoff and Doy 1972).

(h) A large number of ecological variants are available (Redei, 1970, 1974). It can be crossed with several species of different basic chromosome numbers (Laibach, 1958; Kribben, 1965; Berger, 1968; Mesicek 1967). The major advantage of *Arabidopsis* is that it can be subjected to manipulations common to microorganisms which are impractical in most other higher plants.



Plate. 1.1. *Arabidopsis thaliana* (wild type) plants raised under growth-room conditions of constant illumination of $230\text{m}^{-2}\text{s}^{-1}$ light intensity, 271×10^{17} photon photosynthetically active radiation & 25°C temperature.

1.1.3 Cytogenetics

The haploid genome consists of five small chromosomes (Laibach, 1907; Schweizer et al., 1988) and multiply marked strains with mutations on each of the chromosomes are available (Koornneef and Hanhart, 1983). Cytological analysis is cumbersome, because of very small chromosomes (Steinitz-Sears, 1963 and Mesicek, 1967). Sparrow et al. (1972) found that *Arabidopsis* has the smallest nuclear volume among the higher plants and the DNA content of the diploid nuclei is estimated to be 4×10^9 nucleotide pairs or 0.8 pg (Bennett, 1974). Two genetic maps exists, one comprised of more than 80 visible and biochemical mutations (Koornneef et al. 1983; Koornneef 1987), the other of 90 restriction fragment length polymorphism (RFLP) markers (Chang et al. 1988).

1.1.4 Evolutionary trends

Arabidopsis is also an ideal experimental plant from the evolutionary point of view. It can be crossed with its some of related species. Berger (1968) attempted 194 different interspecific crosses with various related genera and obtained viable offspring in 6 combinations. *Hylandra suecica* ($2n = 26$) can be successfully hybridized with *Arabidopsis* ($2n = 10$). *Hylandra* and *Cardaminopsis arenosa* ($2n = 32$) can also produce sterile hybrids (Laibach, 1958; Kribben, 1965). The tetraploid *C. arenosa* does not set viable seed with diploid *Arabidopsis* (Berger, 1968; Mesicek, 1967; Laibach, 1958). Diploid *C. petraea* ($2n = 16$) also yields sterile offspring with diploid *Arabidopsis* (Mesicek). Cytological observations indicate that the genera *Hylandra*, *Cardaminopsis* and *Arabidopsis* share homologous chromosomal regions through some common ancestor(s) (Berger, 1968). The various possible combinations among these species may yield a continuous series of new

chromosomal types of plants (Redei, 1975).

1.2 *In vitro* studies of pollen

1.2.1 Characteristics of pollen germination

The use of pollen grains for experimental studies goes back to the early part of this century, and it is estimated that more than 10,000 articles have been published on the biology of pollen (Vasil, 1987). The discovery of germination of a pollen grain resulting in the formation of a pollen tube was accidentally made by the Italian astronomer and mathematician Giovanni Batista Amici (1824, 1830), in *Portulaca oleracea* by observing some hairs (actually pollen tubes). Von Mohl (1834) reported the first pollen tube growth outside the stigma and observed that pollen grains of certain species grow pollen tubes when placed in a saturated humid atmosphere. Brink (1924a) reported that different species vary in their pollen germinability *in vitro*. Schleiden (1849) and Van Tiegem (1869) made the first attempt to germinate pollen artificially.

Investigators of pollen tube growth biology have historically emphasized either incompatibility response (Heslop Harrison, 1975) or alteration of expected Mendelian ratio as a result of differential pollen tube growth rates (Pfahler and Linskens, 1972; Currah, 1981). In recent years the importance of differential pollen tube growth rates expressed during gametophytic competition in the ecology and evolution of species has been given considerable attention (Mulcahy et al. 1974). Substantial amount of information is thus available on pollen biology, pollen physiology and biochemistry of pollen and has frequently been reviewed (Mascarenhas, 1975).

1.2.2 Formulating germinating medium

The components of artificial media for *in vitro* pollen germination largely depend on the species in question. The elongation of a pollen tube in flowering plants is exceedingly rapid and its requirement, in general, seems quite undemanding, i.e. water, oxygen, and suitable osmotic conditions. Despite extensive attempts to hasten this growth process with the conventional host of growth factors, few have met with convincing success. In many of the angiosperm species pollen grains germinate in sugar solution. The best and the most commonly used source of carbon is sucrose, but lactose (Bishop, 1949; Conger, 1953; Vasil, 1960a, Hrabetova and Tupy, 1964;), dextrose (Faull, 1955; Vasil 1960a, 1962b), raffinose (Hrabetova and Tupy, 1964; de Bruyn, 1966a, 1966b; Roggen and Stanley, 1969), and several other sugars and sugar derivatives have also been found useful (O'Kelly, 1955; Hellmers and Machlis, 1956; Raghavan and Baruah, 1956a; Vasil, 1962b;). Good results were obtained with sucrose, dextrose, rhamnose, raffinose, lactose and galactose, while fructose, mannose and mannitol are mostly unsatisfactory. Investigators have found contradictory evidence for the role of sugar in pollen germination and tube growth. Many of the workers believe that sugars are only needed for osmotic control rather than as a nutritional requirement (Jost, 1905, 1907; Martin, 1913; Anthony and Harlan, 1920; Visser, 1955). The opposing school of thought believes that apart from having an osmotic role, the exogenously supplied sugars, whether *in vivo* or *in vitro*, serve as chief nutritional sources (Brink, 1924a; O'Kelly, 1955, 1957; Hellmers and Machlis, 1956; Vasil, 1960a,b, 1962b, 1964b; Johri and Kessler et al. 1960; Hrabetova and Tupy, 1963;). Jost (1907) while working with pollen of *Hippeastrum aulicum* obtained 17-22 mm long tubes when grown in 1% (w/v) sucrose and only 7-8 mm long tubes in 0.25-0.5% (w/v) sucrose. Pollen of *Nico-*

tiana alata, *Scilla* (Brink, 1924a), *Crotalaria juncea* and *Dolichos lablab* (Vasil, 1964b) also produced long tubes in sucrose solution of suitable concentrations. Chiang (1974) used 16 sugars as carbon sources for cabbage pollen germination. Only six; sucrose, raffinose, lactose, maltose, melizitose, and trehalose supported germination. Sucrose produced the highest percent of germination, while raffinose produced the longer pollen tubes. Harbetova and Tupy (1964) evaluated the effect of different sugar media on the growth of pollen from 49 species. They reported that the pollen tubes of 41 species had best growth on a sucrose medium. Pollen tubes of 7 species grew best on a glucose medium, whereas pollen tube of *Salix caprea* L. grew only on a fructose medium. Pearson (1932) stated that cabbage pollen could germinate in 1% (w/v) glucose but satisfactory germination could be obtained by supplementation of glucose with boric acid. Rashid et al. (1985) achieved germination of *Juniperus* pollen in a medium containing a mixture of 0.5% boric-acid solution and 5% sucrose solution at 18°C.

The role of boron in plant development and its deficiency and toxicity have been extensively studied (Gauch and Duggar, 1954; Loughman, 1961; Lee and Arnnoff, 1967) Aghulon (1910) suggested that the boron may be an essential element that is required for normal development and growth of higher plants, and this was confirmed by the findings of Maze (1915) and Warington (1923). Flowers, especially the tissues of stigma, style and the ovary, often contain high concentrations of boron (Bertrand and Silberstein, 1938; Bobko and Zerling, 1938; Thomas, 1952; Gauch and Duggar, 1954), that play an important role in fertilization. The boron deficiency in pollen of many species has been investigated (O'Kelly; 1957, Linskens and Kroh, 1970). The boron content of pollen is 0.7 ug/mg dry weight, while stigmas may contain 10 times that level (Stanley, 1971). Chiang (1974) used

16 combinations of H_3BO_3 and $CaCl_2 \cdot 2H_2O$, and found that maximum germination percentage was obtained by addition of 50 ppm and 100 ppm of boric acid and calcium respectively.

The role of various growth regulators in pollen germination and tube growth has been studied in recent years (Balasimah and Tewari, 1977). Raghavan and Baruch (1956b) have emphasised that certain auxins like 3-indole acetic acid (IAA), 3-indolebutyric acid (IBA), 3-indole propionic acid (IPA) and 2-naphthaleneacetic acid (NAA), vitamins like para-aminobenzoic acid (PABA), ascorbic acid (AA) and inositol and trace elements like boric acid (H_3BO_3) cobalt chloride ($CoCl_2$), lithium-chloride ($LiCl_2$), manganese sulphate ($MnSO_4$) ammonium molybdate [$(NH_4)_2MoO_4$], zinc sulphate ($ZnSO_4$) and auric chloride ($AuCl_3$) exert a growth promoting action, the stimulation being manifest in an increase in the extent of germination of the pollen grains and enhanced rate of growth of pollen tubes. The positive action of stimulants on the pollen grains has been explained in relation to the auxin/vitamin/trace element balance maintained by these substances naturally occurring in the pollen grains, supplemented by that in the external medium. It is upon this assumption that the theory of pollen tube growth by stimulants has been founded.

Like other factors, calcium has also played an important role in pollen studies. It is widespread and abundant in most flowering plant tissues, averaging around 1.5% of dry weight of leaves, 1.2% in shoots and 0.2% in seeds. Pollen grains are also low in calcium content, averaging about 0.03% (Todd and Bretherick, 1942). Brewbaker and Kwack (1964) concluded that calcium overcomes the population effect, promoting germination and elongation in all pollen types tested (over 100 species), both in culture and *in situ*. They also concluded that the calcium effect

is dependent on the presence of a suitable osmotic milieu, oxygen and borate and is enhanced by methyl donors and other inorganic cations (especially Mg, K, Na, and H). Brewbaker and Kwack (1963) recommended the broad based basal medium (10% (w) sucrose, 100 ppm H_3BO_3 ; 300 ppm $Ca(NO_3)_2 \cdot 4H_2O$; 200 ppm $MgSO_4 \cdot 7H_2O$; 100 ppm KNO_3) which is widely used for experiments on many species.

The first stage in pollen germination is believed to involve uptake of water by the pollen grains (Mascarenhas and Bell, 1969). The moisture of pollen for germination is provided by various stigmatic secretions, but in the *Cruciferae*, because the stigmatic surface is covered by a layer of cuticle, this initial moisture is believed to be obtained from cuticular transpiration (Linskens and Kroh, 1970). The stigmatic surface in leguminous plants generally has an exudate which contains sugars, lipids, amino acids, phenolic compounds and proteins (Heslop-Harrison and Shivanna, 1977). The exudate helps to retain pollen on the stigmatic surface and acts as a medium for pollen grain germination and pollen tube growth (Konar and Linskens, 1966). Turano et al. (1983) reported an exudate on the stigmatic surface of chick pea localized on the most distal cluster of papillate cells of the stigma.

Recently Demeke and Hughes (1991) investigated the effect of sucrose, H_3BO_3 , KNO_3 , $MgSO_4 \cdot 7H_2O$ and $Ca(NO_3)_2 \cdot 7H_2O$ on pollen germination of *Phytolacca dodecandra* L. (endod) in a liquid medium. They recorded that sucrose and H_3BO_3 were critical to pollen germination. A concentration of 10% sucrose and 161.8 μM H_3BO_3 gave over 70% pollen germination. The germination of pollen was not enhanced by $Ca(NO_3)_2 \cdot 7H_2O$, KNO_3 and $MgSO_4 \cdot 7H_2O$

Cheng and Comb (1992) reported 81.70% pollen germination in wheat when

pollen cultured on 0.7% agar medium supplemented with $100 \text{ mg l}^{-1} \text{ H}_3\text{BO}_3$, $300 \text{ mg l}^{-1} \text{ CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 0.75 M raffinose.

1.2.2.1 Pollen tube growth

In general the rate of pollen tube growth is directly proportional to the length of style, i.e. pollen tubes grow slower in a short style than in long a style. It is possible that in short styles the cell layers are more compact, thus preventing rapid growth of pollen tubes (Bassiri, et al., 1987). Lehman and Purj (1967) studied *in vitro* rate of germination and tube growth of fresh and stored pollen. First germination occurred in all samples after about 10 minutes of incubation. With fresh pollen maximum germination was reached at 1-2 hrs. Stored pollen which varied in age from 42 to 101 days was different in response and germinated slowly and required longer times to reach maximum germination. They also reported highest average correlation between tube length and time (correlation coefficient of 0.95) and lowest between tube length and germination. De Beer (1963) working with pollen of pea nut (*Archas hypogaea L.*), observed the first sign of germination about 9 minutes after sowing in an agar medium. Growth was at a rate almost proportional to time for the first 50 minutes. Hoekstra (1983) observed that some pollen tubes of *Geranium maculatum* reached the ovary within 30 minutes of germination. Their actual growth rate through the 4 mm styler tissue is therefore at least 0.133 mm/min., fairly typical for trinucleate pollen.

In vitro growth curves of pollen tubes have been described as sigmoid for many species (Vasil, 1960a, 1962b;). These are categorized in three phases. The first is a lag phase of slow growth, followed by rapid elongation period and finally a period of gradual decrease in growth resulting in stoppage of tube growth. Brewbaker

and Majumder (1961) described how growth patterns of pollen populations show a brief lag phase, a linear growth phase and a final leveling out phase. They emphasised that under optimal conditions individual pollen tubes grow at a linear rate. Ottaviano et al. (1980) stated that the speed of pollen tube growth is positively correlated with the quality of the resultant sporophytic generation. Therefore gametophytic competition may be an important adaptive mechanism. Furthermore pollen tube growth rates may be used to predict the quality of F_1 crosses in crop species.

1.2.2.2 Pollen growth rates

The time period from pollen germination to ovule penetration by the pollen tube differs greatly in different species, some of which are listed as under.

Species	Time period from pollen germination to ovule penetration	Reference
Apple (crosses)	4 d	Stott, 1972
Apple (selfed)	8-12 h	Stott, 1972
<i>Avocado</i>	24 h	Tomer and Gottreich, 1975
<i>Medicago</i> (interspecific crosses)	20-30 h	Sanguen, et al., 1983
<i>M. disciformis</i>	15-22 h	Sanguen, et al., 1983
<i>M. rigidula</i>	12-24 h	Sanguen, et al., 1983
<i>M. scutella</i>	12-19 h	Sanguen, et al., 1983
<i>Pearl millet</i> and <i>Zea mays</i>	8 h	Reger and James, 1982

With few exceptions, *in vitro* pollen tube growth rate is much slower than that

in vivo. Only in a few instances does the *in vitro* pollen tube length equal that in natural conditions, in *Rumex* (Schoch-Bodmer, 1921), *Pyrus* (Knight, 1917, Harabetova and Tupy, 1964), *Chionodoxa*, *Hippeastrum*, *Muscari*, *Puschkinia*, *Scilla* (Brink, 1924c), *Vitis* (Branscheidt, 1929, 1930), *Convallaria*, *Echeveria*, *Gagea*, *Genista*, *Impatiens*, *Pachyphyllum*, *Ribes*, *Scilla*, *Sedum*, *Tradescantia*, *Vicia*, *Vinca*, *Xanthosoma* (Ehlers, 1951) and *Pennisetum typhoideum* (Vasil, 1960a). In most of the species the *in vitro* tube length may be 10% of that of pollen tubes *in vivo*. This strongly supports the idea that pistil tissue provides a much more suitable milieu for the rapid growth of the pollen tube than that provided *in vitro*.

The search for various factors enhancing *in vitro* pollen tube growth is continuing. In the case of *Tradescantia paludosa*, not only do pollen tubes reach the length of those produced in nature but these even grow at about same rate in liquid suspension culture as in the style and in most other respects their growth *in vitro* is comparable to that *in vivo* (Mascarenhas, 1966a). Rate of pollen tube growth *in vivo* is in the range of 1500-3000 $\mu\text{m}/\text{h}$ in most species with a maximum of 7500 $\mu\text{m}/\text{h}$ (Buchholz and Blakeslee, 1927; Brewbaker and Kwack, 1964). Poddubnaja-Armoldi and Dianowa (1934) reported an incredible rate of 35000 $\mu\text{m}/\text{h}$ for *Taraxacum kok-saghyz* *in vitro*. In comparison, pollen tubes growing *in vitro* in this species elongate at the rate of only 100-300 $\mu\text{m}/\text{h}$. Vasil (1960a) reported rates in excess of 2000 $\mu\text{m}/\text{h}$ for pollen tubes of *Cucumis melo* *in vitro*.

1.2.3 Effect of pH

Opinions on the effect of pH are so conflicting that it is difficult to decide whether it influences the germination of pollen at all (Vasil and Bose, 1959). Gotoh (1931) was first to point out that dissolved alkali of the microscope slide cover

glass greatly affected pollen germination . Since then many workers reported pH levels for different plant species. In *Petunia*, best results were obtained between pH 5.5 and 5.8. Brewbaker and Majumder (1961) reported optimum germination of *Petunia* pollen between pH 5.0 and 7.5, with severe inhibition below pH 4.5. Brink (1925) reported pH 7.0 as optimum for pollen germination of *Lathyrus odoratus* and mentioned that the range of favourable pH for germination is rather narrow. Double optima curves (two-peaked) expressing the percentages of germination in a wide range of pHs have been reported for several plants (Berg, 1930; Branscheidt, 1930). In many other plants satisfactory germination of pollen takes place within a pH range of 4-9 (Berg, 1930; Branscheidt, 1930; King and Johnston, 1958; Goss, 1962). Vasil and Bose (1959) did not find any change in the pH of the medium even after pollen tubes had grown in it for about 2 hours. The optimum pH range suitable for *Cosmos* pollen changes about every 20 days during the flowering season (Kubo, 1954). Pollen grains of several species of *Rhododendron* germinated at pH 3.0 at the beginning and the end of flowering season, but will germinate anywhere between pH 1.8 and 7.8 during the peak flowering season (Kubo, 1955a,b). The optimum pH for germination and growth in media containing borate compounds is generally between 6.5 to 7.1 (Stanley and Lichtenberg,1963). In some species the percentage of germination and the length of pollen tubes remain fairly constant between pH 3.5 and 9.0 (Vasil and Bose 1959). Leduc et al. (1990) tested pollen germination of *Capsella bursa-pastoris* at pH 3.0-9.0, and reported an optimum germination (30%) at pH 8.0, and pH ranging from 5.0 to 7.0 produced 17-21% pollen germination respectively. Extreme pH conditions of 3.0 and 9.0 were reported to be inhibitory.

1.3 Effect of temperature

1.3.1 Effect of temperature on pollen germination

The correlation between temperature and pollen germination is usually represented by an optimum curve and the Q₁₀ for pollen tube growth is approximately 2 (Roberts and Struck Meyer, 1948; Visser, 1955; Vasil, 1962b). While working on maize Maria and Johnson (1981) suggested that prolonged exposure to temperatures above 32°C can reduce pollen germination of many genotypes to levels near zero. In some species germination is believed to be influenced by age and ripeness of the pollen, as well as temperature before and during anthesis (Hayas, 1955). Weinbaum et al. (1984), reported maximal pollen germination percentage at 16°C and 23°C respectively for almond and peach. Pollen grains of *Tradescantia* respond to heat treatment by adaptation, but not by synthesise heat shock proteins (HSPs) (Mascarenhas, 1984; Xiao and Mascarenhas, 1985). This suggests that pollen do react to high temperatures, but in different ways to vegetative tissue. In contrast to vegetative tissue (Schlesinger et al. 1982; Munro and Petham, 1985), the reaction of pollen to heat stress has been investigated (Mascarenhas and Altschuler, 1983; Cooper et al., 1984). This is remarkable, since high temperatures during pollen maturation and pollination adversely affect seed yield (Mascarenhas, 1975, 1984). At low temperatures the germination percentage and tube length are considerably reduced and under such conditions *in vivo* the tubes may never reach the ovules (Smith and Cochran, 1935). Between 90 to 100 % germination of apple pollen takes place at 14°C after 24 hours (Adams, 1916).

1.3.2 Effect of temperature on pollen tube length

Pollen tube growth is sensitive to temperature (Brink, 1924a,b,c). At sub

optimal temperatures satisfactory tube length can be obtained only if the period of the growth is not a limiting factor. The diameter of pollen tubes increases with increasing ambient temperature (Smith, 1942). In several plants marked swelling of pollen tube tips, branching and bursting have also been observed when pollen grains are cultured at temperatures higher than 40°C (Vasil and Bose, 1959; Vasil, 1962b). Temperature therefore seems to affect the rigidity of the pollen tube wall. The mechanism by which it does so is not understood (Vasil, 1987). Ahmadi (1956), Inoui and Suzuki (1959), and Dickson and Boettger (1984a) indicated that in beans decreased pod set at high temperatures is related to pollen injury, but Halterlein et al. (1980) reported that a constant 35°C actually increased pollen production and did not decrease the number of pollen tubes reaching the base of the style. Iwami (1951) reported a highly negative correlation between percentage pod set and temperatures in beans.

1.4 Impact of differential pollen growth rates on the sporophytic traits

Differential rates of pollen tube growth have been described in several plant species (Harding and Tucker, 1969). Genes expressed by microgametophytes that contribute fast-growing pollen tubes may also facilitate more vigorous sporophytes (Mulcahy, 1974; Ottaviano et al., 1980; Winsor, et al., 1987). Consequently pollen tube competition may result in increased seed "quality" because the fastest-growing pollen-tubes are most likely to fertilize ovules. Snow and Spira (1991) using self-compatible *Hibiscus moscheutos* studied the potential for non-random fertilization by comparing growth rates of pollen-tubes from different donors. Relative pollen-tube growth rates were determined by applying pollen from pairs of donors to different stigmas on adjacent stylar branches. They measured the number of callose plugs per tube in cross-sectional transects across the style after 3 hr.

They demonstrated that rates of callose plug formation can be used as a sensitive indicator of relative pollen-tube growth rate. They concluded that variation in pollen-tube growth rates leads to non-random paternity within fruits.

Correlations between gametophytic and sporophytic growth rates may be due to overlapping gene expression during the two phases of a plant's life cycle (Mulcahy, 1979; Tanksley et al., 1981; Willing and Mascarenhas, 1984). Evidence of correlation between gametophytic and sporophytic qualities could be useful because gametophytic competition is both frequent and severe in natural populations (Hartl, 1970). A statistically significant correlation has been reported by Mulcahy (1971) in *Zea mays* between the relative speeds with which pollen tubes penetrate the lengths of styles and the relative competitive abilities of the resultant zygotes. Mulcahy (1974) reported that speed of pollen tube growth is positively correlated with the quality of the resultant sporophytic generation. Therefore, gametophytic competition may be an important adaptive mechanism. The pollen tube growth rates might be used to predict the quality of F1 crosses in crop species.

When mixtures of types of corn pollen, identified by genetic markers, were applied to the silks of other inbred lines, the rate of pollen tube growth often varied with type of pollen. This gametophytic differential growth is correlated with sporophytic differential vigor. Relatively heavier seeds in seed mixtures resulted from fertilization by gametes from faster growing tubes. The increased seed weight is due to the greater competitive ability of the zygotes thus formed (Mulcahy, 1971).

1.5 Male gametophyte growth: an index for prediction of salinity limits and selection for salt tolerance in plant species

Approximately 33% of irrigated land world wide is affected by salinity (Carter, 1975) and the problem is becoming severe in many areas (Chauhan, 1987). More and more marginal land (including salt affected soils) will have to be used for crop production because of increasing demands of population. Conventional methods of ameliorating salt-affected soil through reclamation, drainage, and excess use of irrigation water to leach salts below the root zone have to be complemented by the genetic approach through screening and breeding crops for higher salt tolerance (Ashraf and McNeilly, 1990; Epstein and Rains, 1987; McNeilly, 1990; Yeo and Flowers, 1986). Genetic and physiological approaches should merge into a unifying, more comprehensive approach to dealing with soil salinity problems (Blum, 1988).

Saline waters contain many different dissolved minerals, but sodium and chloride ions usually predominate. The inhibition of growth is often correlated with either *Na* or *Cl* uptake. Cultivars within species that are more salt-tolerant often exclude more *Na* (Flowers and Yeo, 1981; Hajibagheri et al., 1987; Schachtman et al., 1989).

Growth of halophytes, particularly dicotyledonous species, is generally stimulated by intermediate salt levels (Flowers et al., 1977). Under saline conditions, leaves of halophytes accumulate *NaCl* for osmotic adjustment, and often increase in succulence (Flowers and Yeo, 1986).

Salinity effects are found in every part of a plant body.

1.5.1 Effect of salinity on reproductive parts

Few accounts of the impacts of salinity on various stages of reproductive growth have been published.

Adverse effects on corn grain production (Piruzyan, 1959) and at rice grain setting (Kapp, 1947) have been reported. These results indicate that salinity disrupts reproductive growth of these plants. Ota et al. (1956) demonstrated that a reduced number of fertilized florets in rice panicles under saline conditions is due to the possible effects of salts on pollen viability and germination. Abdullah (1978), working with wheat found that the increasing salinity levels decreased the pollen viability and germination, while the number of seeds per ear was not reduced. Sacher et al. (1983) observed in *Lycopersicon* that the germination of saline-grown pollen on saline medium was greater than control grown pollen on saline medium. They also reported in *Lycopersicon* that mean dry weight of saline pollinated seed was significantly greater than that of plants from controlled pollinated seed.

1.5.2 Effect of salinity on roots

The primary effect of salinity is thought to take place in roots (Meizner, et al. 1991; Munns and Termaat, 1986). It was argued that this primary effect is a water deficit effect rather than a specific toxic effect of salt *per se* (Munns and Termaat, 1986). In the short-term responses of whole plants to salinity, water status of roots regulates the shoot growth through a messenger system that is likely to include growth substances (Munns and Termaat, 1986). One candidate for this putative hormonal signal is ABA (Kefu, et al., 1991; Saab and Sharp, 1989; Zhang and Davies, 1989). Kramer (1988) argued in favour of direct hydraulic effect being responsible for shoot salt stress contrary to the concept of biochemical a signal.

Rapid decreases in root elongation of maize seedlings were observed if high sodium and lower calcium salts were applied, but short-term elongation rates were largely restored by the application of calcium (Cramer et al., 1988). In contrast to studies conducted in hydroponics, results from experiments using soil-grown plants have often shown at least as severe reductions in root as in shoot growth at elevated salinities (Bingham and Garber, 1970; Chavan and Karadge, 1980; Papadopolous and Rending, 1983; Zekri and Parsons, 1990; Snapp et al., 1991). This suggests that salinity effects on root systems can be significant and may be affected by the growth media. Marcum and Murdoch (1992) reported the root growth stimulation at salinities up to 450mM *NaCl* in *Sporobolus virginicus* (L) Kunth.

1.5.3 Effect of salinity on shoot

In contrast to ideas that roots are the primary sensors of salt toxicity, Cramer and Bowman (1991) found that short-term *Zea mays* leaf elongation is independent of the roots. Alternatively it was suggested that nutrition of the shoot apical meristem may be disturbed in the initial stages of salt stress and that the shoot meristem might be the source of a signal to expanding leaves (Lazof and Lauchli, 1991).

An inhibition of shoot growth as a long-term response (weeks and months) would result from excessive accumulation of salt in leaves (Flowers et al., 1991; Yeo and Flowers, 1986; Yeo, et al., 1991). This accumulation results in osmotic and ionic effects (high salt concentration in the apoplast of leaf cells causes a large water deficit in symplast if the uptake and compartmentalization of salt is not as fast as the transport of salt from roots) that both appear to be important in the long term response to salinity. Since the volume of the apoplast of leaf cells is

very small (Tomos, 1988), only a small amount of ions is required to significantly increase the apoplast concentration, and therefore severely affect water relations in the leaf cells (Yeo et al., 1991).

Marcum and Murdoch (1992) working with *Sporobolus virginicus* (L) Kunth suggested that shoot growth was stimulated by intermediate salt levels, concurrent with both an accumulation of *Na* and *Cl* in shoots and a slight increase in shoot succulence.

1.6 Metal tolerance

We are just begining to understand the effects that metal ions have in biological systems. This area of science has so many aspects that it easily lends itself to a multidisciplinary approach. Chemists are exploring the molecular aspects of metal ions binding, molecular biologists are investigating how metal ions are involved in gene regulation, while other biologists are studying population and species differences in metal ion tolerance. It is only through the interactions of scientists from different backgrounds that we will be able to completely determine the roles of metal ions in biology (Borovik, 1989). Several heavy metals and metalloids, such as iron, manganese, zinc, copper and molybdenum are essential micro nutrients, whereas others such as aluminium, cadmium, arsenic, uranium, lead, thallium, chromium, mercury, silver and gold are not. Nickel, cobalt and vanadium may be essential for a limited number of species (Ernst, 1982). Enrichment of heavy metals in soils, either through such natural processes as geochemical anomalies or through human activities (utilizing mineral resources), usually coincides with increased resistance levels in plants (Antonovics, 1971). Metal resistance is typically a quantitative characteritic (Urquhart, 1971), correlated with the prevailing metal

availability levels in the soil (Antonovics. 1971).

Two adaptations are generally described for heavy metal resistance i.e. by avoidance and by tolerance. Levitt (1980) defined avoidance as an organism's ability to prevent excessive metal uptake in its body. Tolerance is an organism's ability to cope with metals that are excessively accumulated within in (some part of) its body. Metal resistance is usually highly metal specific (Ernst, 1982), and confined to those metals that are enriched in the soil. Multiple resistance is combined resistance to several metals and is often associated with co-occurrence of high levels of these metals in the soil (Gregory and Bradshaw, 1965). This phenomenon has been explained as resulting from cross-resistance or co-tolerance, ie resistance achieved by a mechanism to cope with excessive levels of another metal (Cox and Hutchinson, 1980, Walley et al., 1974).

1.6.1 Tolerance mechanism

Like other organisms, higher plants are also believed to possess intercellular metal buffer systems i.e. metal chelating substances, which serve to keep the intercellular availability of essential metals within certain limits. Plants accumulate many metal-chelating compounds upon exposure to excessive metal availability levels, such as amino acids and amino acid derivatives (Ernst, 1982; Peterson, 1981), citric acid (Lee et al., 1977; Thurman and Rankin, 1982), malic acid (Ernst, 1975 and Brookes et al. 1981) and phytochelatins (Grill et al., 1985, 1987; Verkleij et al., 1988). It is often suggested that metal tolerance may depend on the capacity and the nature of metal buffer systems (Steffens et al., 1986; Scheller et al., 1987). Selenium tolerance probably depends on the ability to bind the selenium as selenomethyl-selenocysteine or selenocystathionine, instead of a selenomethion-

ine and selenocysteine, which are accumulated in sensitive species (Rosenfeld and Beath, 1964). On the other hand, sensitive and tolerant plants produce similar phytochelatins after exposure to elevated levels of heavy metals (Verkleij et al. 1988). There is only limited evidence for quantitative differences in the capacity of phytochelatin production between sensitive and tolerant plant cell cultures (Scheller et al., 1987; Jackson et al., 1987). For intact plants there is no evidence at all for such quantitative differences (Robinson and Thurman 1986). Moreover, increased phytochelatin synthesis can be induced by almost any metal (Grill et al., 1987).

1.6.2 Effect of metals on pollen germination and growth

The consequences of calcium on the pollen has already been reviewed. A few metals and other compounds known to stimulate pollen growth are listed below.

Effect of copper

Allen and Sheppard (1971) while studying copper tolerance in Californian populations of the monkey flower (*Mimulus guttatus*) concluded from tests on F1 progeny of crosses involving tolerant and non-tolerant plants that copper tolerance is dominant in effect at low copper concentrations, intermediate at intermediate concentrations and recessive in effect at high copper concentrations. Populations of *Agrostis tenuis* Sibth, tolerant to normally toxic levels of lead, zinc, copper or nickel have been described by Bradshaw (1952), Wilkins (1957) and Jowett (1958). McNeilly and Bradshaw (1968) demonstrated that *A. tenuis* tolerant and nontolerant clones differed in their response to a range of copper concentrations in solution. Gregory and Bradshaw (1952) calculated a correlation coefficient of 0.64 between index of tolerance for *Cu* and total *Cu* content in *A. tenuis*.

List of some compounds stimulating pollen growth as reported by some earlier pollen researchers.

Compounds	Reference
Amino acids	Sawada, 1958
Antibiotics	Brewbaker and Kwack, 1963; Bose, 1959
Auxins	Loo and HWang, 1944; Raghavan and Baruah, 1956b; Sen and Verma, 1963; Vasil, 1960
Boron	O'Kelly, 1957; Schmucker, 1933; Visser et al., 1955
Cobalt	Yamada, 1958
Colchicine	Loo and Hwang, 1944; Sen and Verma, 1963
Gibberellin	Bose, 1959; Chandler et al., 1957; Kato, 1955
Kinetin	Bose, 1959
Manganese	Loo and HWang, 1944;
Plant tissue extracts	Brink, 1924; Loo and HWang, 1944; Sen and Verma, 1963
Sodium and potassium	Brink, 1924
Yeast extracts	Faull, 1955

Greater root accumulation by tolerant genotypes has been found for copper in *Agrostis stolonifera* (Wu, et al., 1975; Wu and Antonovics, 1975). Macnair (1983) reported that *Cu* tolerance in *M.guttatus* was determined primarily by a single major gene.

Effect of aluminium

Cotton (*Gossypium hirsutum* L.) root growth is often restricted in acid soils due to toxic levels of soluble aluminium throughout the soil profile (Adams and Lund,

1966, Soileau et al., 1969). Liming top soils improves root growth by decreasing the solubility of Al (Adams 1986). Kennedy and Smith (1986), working with cotton species, found that root growth was reduced 80% or more by 2 or 10 mg Al liter⁻¹ for all genotypes tested. Searcy and Mulcahy (1990) working with comparative toxicity of aluminium in tomato cultivars, considering root growth as a criterion of sporophytic Al sensitivity, produced results similar to pollen germination.

Aluminum application under boron starvation promotes growth of tea (*Camellia sinensis*) pollen tube (Konishi, 1980) and root (Konishi, 1981). Aluminum tolerance is based on nuclear genes (Lafever and Campbell, 1978; Rhue et al., 1978) so that it could be selected for in pollen and it tends to be specific for that metal (Foy et al., 1973a). Aluminium interferes with many functions that are found in both stages of plant life cycle. Aluminium can interfere with mitosis (Clarkson, 1969), with calmodulin (Haug, 1984), and reduce cell wall extensibility and nutrient uptake (Foy et al., 1978). *In vitro* pollen tube growth (Konishi and Miyamoto, 1983) and germination (Cox, 1986) are sensitive to Al. *In vitro* cell culture studies (Meredith, 1978a,b; Conner and Meredith, 1985a,b) indicate that Al stress can be studied and selected for at the cellular level.

Effect of cadmium

Differential cadmium accumulation occurs in various plant parts, with roots revealing the greatest concentrations, followed by leaves, stems, fruits and seeds (Jastrow and Koeppel, 1980; Weigel and Jager, 1980; Page et al., 1981). Studies carried out in crops revealed that plant species varied with a wide range of cadmium tolerance and in accumulation of this metal (Jastrow and Koeppel, 1980; Page et al., 1981). Miles and Parker (1979) reported shoot biomass reductions ranging

from 18 to 98% for several Midwest U.S. plant species grown in soil for 6 weeks at a *Cd* concentration of 30 $\mu\text{g/g}$. Page et al. (1972) recorded in vegetables growth reductions of 50 to 85 % when raised under Cd-stress of 90 μM from 3 weeks. Hardiman and Jacoby (1984) demonstrated that significantly higher tissue Cd concentrations occurred in *Phaseolus vulgaris* under conditions conducive to high transpiration rates. Wong et al. (1984) in *Brassica chinensis* showed 35 and 95 % reductions in whole plant fresh weight after a 25 day exposure at solution Cd concentrations of 9 μM and 90 μM , respectively. A 50 % growth reduction was observed in *Brassica oleracea* after a 3 week exposure to a solution Cd concentration of 90 μM , while leaf tissue Cd concentration was measured at 7.3 $\mu\text{Mol./g}$ dry weight (Page et al., 1972).

The effects of cadmium on tissue concentration of several elements have been reported in the literature; however, the results are often contradictory as Cd-plant nutrient interactions are complex and influenced by environmental and plant physiological factors (Jastrow and Koeppe, 1980; Page et al., 1981). Root et al. (1975) reported that in hydroponically grown corn the concentration of *Fe* increased as the *Cd* concentration increased, whereas, John (1976) recorded decreases in *Fe* and *Mn* as *Cd* supply was increased in hydroponically grown Lettuce (*Lactnia sativa*). Turner (1973) reported that enhanced *Cd* supply increased *Zn* in the shoots of several vegetables, whereas Root et al. (1975) observed decreased concentrations in the leaves of corn as tissue *Cd*-increased. In *Fe*-stress experiments conducted in vermiculite-hydroponic double-container plot culture systems, Tingy et al. (1982) observed a significant increase in *Mg*, *Fe* and *Al* in *Arabidopsis thaliana* foliage.

Thomas et al. (1986), studying direct and residual effects of cadmium on the growth and elemental composition of *Arabidopsis*, reported that the biomass in the

first generation plants decreased in response to nutrient solution containing increasing *Cd*-concentrations. The 100 μM *Cd* treatment significantly reduced rosette, raceme and mature seed biomass. Additionally, enhanced *Cd* concentrations were observed in rosette, raceme and seed tissues. However the seed concentrations were substantially less than the rosettes of first generation plants, but revealed significant increases in *Mg*, *S*, *Cu*, *Fe*, *Mn* and *Al*. Significant decreases in *N* and *P* were observed in the racemes and there was significant loss of *Ca* in the seeds. Subsequently, second generation plants (germinated from seeds collected from the first generation plants) were similarly cultured except that *CdCl*₂ was omitted from the nutrient solution. The progeny from the first generation plants revealed no significant residual effects as far as growth and elemental composition are concerned.

Effect of zinc

Early work of Turner and Marshal (1972) on the physiology of tolerance suggested that zinc was bound up in the cell wall of zinc tolerant plants, thus preventing the metal from inhibiting cellular enzyme systems. Wainwright and Woolhouse (1977), however, argued that this may not be the only mechanism, and showed that individual enzyme systems may show tolerance. Evidence has been advanced for a compound that binds zinc in the roots of zinc-tolerant plants (Turner, 1973; Peterson, 1981; Wyn Jones et al., 1971). The nature of zinc tolerance in higher plants is poorly understood. Woolhouse (1983) has proposed that the tolerance mechanism depends primarily on aspects of internal compartmentation rather than on exclusion mechanisms.

Godbold et al., (1984) working on an accumulation of zinc and organic acids

in roots of zinc tolerant and non-tolerant ecotypes of *Deschampsia caespitosa* concluded that, a much higher *Zn* level is necessary to inhibit root elongation in the zinc tolerant ecotype compared with a non-tolerant ecotype. In the presence of a range of high levels of zinc, it accumulated to a much higher concentration in the roots of the tolerant ecotype, especially in the root sap.

1.7 Anatomical changes brought about by salinity in stem, leaf and root of *Arabidopsis thaliana*

Efforts are continuing to develop economically important salt tolerant plant species which may yield more. It has long been known that soil salinity is a major concern in the agriculture of arid and semi-arid regions. According to an estimation one-third of the world's land surface is arid or semi-arid (4.8×10^9 ha), out of which one-half is estimated to be affected by salinity (Croughan and Rains, 1982).

It is widely accepted perception that soil salinity generally, if caused by sodium chloride, adversely affects the plant growth, yield, morphology, anatomy, ultra-structure and metabolism of plant species (Solomon et al., 1986; Mladenova, 1990).

How does salinity induces changes in plant anatomy? A brief review of the literature is cited here under.

Serrato et al. (1992) reported that plants raised under 200 mM *NaCl* had an epidermis made up of smaller cells with thicker mesophyll having more cell rows than the control plants. They also found palisade parenchyma on both sides of the mesophyll and spongy parenchyma made up of cells slightly different from the control. In another studies Serrato et al. (1991) while studying leaflets of *Prosopis tamarugo* Phil. from the plants grown in 400 mM *NaCl*, found larger number of

intercellular air spaces. A progressive decrease in cell size of leaflets as salinity rose was also demonstrated.

Peter and Andre (1987) working on *Hibiscus cannabinus* remarked that the reduction in leaf area was primarily due to the smaller epidermal cell size. The epidermal cell numbers were also significantly reduced by salinity.

The anatomical basis for inhibited leaf growth under saline conditions has been studied in a number of species. Cotton epidermal cells (Ivanitskaya, 1962), and bean spongy mesophyll cells (Wignarajah et al. 1975) showed a decrease in number but not in size under saline conditions. Meiri and Poljakoff-Mayber (1967), reported an increase in epidermal cell number and decrease in epidermal cell size in salinized bean plants. Both cell size and cell number were reduced in unselected tobacco cell cultures grown in salinized media (Dix and Street, 1975).

Serrato et al. (1991) reported an increase in stem diameter of *Prosopis tamarugo* seedlings grown in an increasing salinity (up to 400 mM); this increase in diameter being correlated with higher number of cortex layers. Early lignification is frequently observed in halophytes and in non-halophytes grown in salt, eg. *Silene maritimum* (Miller, 1934), *Arthrocnemum fruticosum* (Saadeddin and Doddema, 1986) and *Pisum sativum* (Solomon, et al., 1986).

1.8 Anatomical changes brought about by certain heavy metals in stem, leaf and root of *Arabidopsis thaliana*

1.8.1 Anatomical changes brought by cadmium sulphate

Cadmium is considered to be a major environmental pollutant and its phytotoxicity is well established (Page et al., 1972;). Cadmium as opposed to other heavy metals like aluminium (Bennett et al., 1984) or chromium (Vazquez et al., 1987) appears to cause more marked ultrastructural changes in the aerial parts of plants than in roots. This may be explained by both the capacity of roots to store *Cd* in a more inactive form, and the relatively high mobility of cadmium within plants.

Several authors have found that *Cd* toxicity caused water stress by inhibiting water uptake and transport (Lamoreaux and Chaney, 1978; Fuhrer et al., 1981).

Inhibition of root growth is an early effect of heavy metal toxicity in plants (Barcelo and Poschenrieder, 1990). Long-term exposure of whole plants to cadmium may affect chlorophyll synthesis and thus have an important role in both, the chloroplast development in young leaves and the inhibition of photosynthesis (Stobart et al., 1985). Vazquez et al. (1992) working with beans (*Phaseolus vulgaris*) recorded a significant decrease in a root elongation after 48 h exposure to cadmium. During light microscopy they observed a decrease in the number of cortical parenchyma cells after 120 to 144 h exposure to cadmium. They found root diameter unaffected due to an increase of cell size. They reported an increase in pericycle cell number and less cell differentiation in vascular cylinder of cadmium treated plants against control.

Barcelo et al. (1988) reported that cadmium treatments after unfolding of primary leaves, reduced the cell size and intercellular spaces. Cell growth primarily depends on the turgor potential as the driving force of the leaf expansion. Cadmium affects both turgor potential and cell wall elasticity (Barcelo et al., 1986b)

1.8.2 Anatomical changes brought by aluminium

The levels of aluminium at which toxicity symptoms appear depends on the plant species, its age and the experimental conditions employed. Structural and functional damage in the roots affect nutrient uptake, leading to reduced growth and mineral deficiency in shoots and leaves. Roots in an advance stage are stubby, swollen, frequently dark brown, and often have rough, ridged surfaces, indicating the deterioration of the epidermis and cortex (Hutchinson et al. 1986; Jorns and Hecht-Buchholz 1985; Metzler and Oberwinkler 1986). McQuattie and Schier (1990) observed the anatomical changes caused by the aluminium-stress conditions in red spruce seedlings. They noted that aluminium decreased the root length but increased the root diameter and the number of cell layers in the root cap. The light microscopy of root sections showed that aluminium-stress caused loss of cells from peripheral cell layers and formation of intercellular spaces. Hecht-Buchholz Hecht Buchholz et al. (1987) stated that accumulation of phenolic compounds in roots of *Al*-stressed seedlings is commonly observed.

1.8.3 Anatomical changes brought by zinc sulphate

Zinc is an essential micronutrient for plants. It is an active component in many metabolic reactions and is known to be compatible with many other metal ions. Effects of zinc on the plant growth are well studied, but considerably less work has been carried out regarding the effects of it on the internal structure of plant

species. Paivoke (1983) studying the effects of zinc on the root anatomy of the *Pisum sativum* reported that the epidermis of Zn-treated roots was lignified and the endodermis also possessed lignified cells. Robb et al. (1980) stated that zinc treatment enhances the synthesis of phenolic compounds in plants.

1.9 Objects of present study

Arabidopsis thaliana, a small flowering weed plant with short generation period & other unique qualities is considered as excellent model for plant research was used to carry out following experiments:

- (1). **Formulating pollen germinating medium.**
- (2). **Effect of temperature on pollen germination and pollen tube growth.**
- (3). **Impact of differential pollen tube growth rates on the sporophytic traits.**
- (4). **Male gametophyte: an index for prediction of salinity limits & selection for salt tolerance in plant species.**
- (6). **Fruit-setting, seed-setting and seed-filling in response to high salinity (*NaCl*) conditions.**
- (7). **Anatomical changes brought by salinity (*NaCl*) in the stem, leaf & root.**
- (8). **Fruit-setting, seed-setting and seed-filling in response to high metal concentrations.**
- (9). **Anatomical changes caused by heavy metals in the stem, leaf & root.**

Chapter II

MATERIAL AND METHODS

2.1 Biological material

The seed of the following genotypes of *A. thaliana* used for experimentation was obtained with the courtesy of my supervisor, Dr. Phillip J. Gates, Lecturer, Department of Biological Sciences, University of Durham, England (UK):

NW-28:- Known as the *apetala* mutant (locus *ap1*), having either no petals or rudimentary ones. Landsberg (Ler) background. No special growth requirements. Mutagen generated with ethyl methane sulfonate (EMS). (The Nottingham *Arabidopsis* stock centre, seed list, Jan. 1992, p-8)

NW-45:- The mutant known as *clavata* (locus *clv1*) with club like siliquas. Ler background. No special growth requirements. Mutagen generated with EMS (NASC, seed list. Jan. 1992, p-12).

NW-46:- The mutant known as *clavata* (locus *clv2*) having club like siliquas. Ler background. No special growth requirements. Mutagen generated with EMS (NASC, seed list Jan. 1992, p-12).

NW-91:- The mutant having a yellow inflorescence (locus *yi*), yellow flower buds and yellow greyish sharper leaves. Ler background. No special growth requirements. Mutagen with EMS (NASC, seed list, Jan. 1992, p-7)

CAD1:- A cadmium sensitive line screened out from wild-type (vari.-Columbia),

by Dr. Ross Howden and Dr. Christophers. Cobbett, Department of Genetics, University of Melbourne, Parkville, Australia, 3052. This genotype was obtained with co-operation of Dr. Nigel Robinson, Lecturer, Department of Biological Sciences, University of Durham.

Wild type (Landsberg erecta)

M2 (Landsberg erecta):- Sub-populations of parental group nos. 28-33. Mutagen treated with the (EMS).

M2 (Columbia group):- Sub-populations 17/60, 18/60, 19/60, 20/60, 21/60, 22/60, 23/60, 24/60, 25/60. Mutagen with EMS.

2.2 Plant Culture medium

In vitro

The following *in vitro* culture medium was used as recommended by Lother Willmtzar's Laboratories, AM, half strength Murashige and Skoog (MSS) Macro and Micro salts (Jonathan, 1990). The details are listed in the Table-2.1.

Table-2.1: The Murashige and Skoog solution.

Name of salt	Concentration (mg l ⁻¹)
<i>CaCl₂.H₂O</i>	219.5
<i>CoCl₂.6H₂O</i>	0.0125
<i>CuSO₄.5H₂O</i>	0.0125
<i>FeNaEDTA</i>	18.35
<i>H₃BO₃</i>	3.1
<i>KH₂PO₄</i>	85.0
<i>KI</i>	0.415
<i>KNO₃</i>	950.0
<i>MgSO₄.7H₂O</i>	185.3
<i>MnSO₄.4H₂O</i>	11.15
<i>Na₂MO₄.2H₂O</i>	0.125
<i>NH₄NO₃</i>	825.0
<i>ZnSO₄.7H₂O</i>	4.3

2.3 Microscope specifications

A Nikon Diaphot-TMD inverted microscope with TMD-EF epifluorescence provided with violet (V), blue violet (BV), ultra-violet (UV) excitation filter cassettes, was used during studies.

2.4 Image analyzer

The microscope mentioned above was coupled with a **Micro Scale TM/TC** a **Image Analysis systems** (Digithurst Ltd. England).

Use of image analyser

Table-2.2 List of chemicals used in experimentation.

Name of chemical	Name of supplier
H_3BO_3	BDH Chemical Ltd., England
$Ca(NO_3)_2 \cdot 4H_2O$	BDH
$MgSO_4 \cdot 7H_2O$	BDH
KNO_3	BDH
$Al_2(SO_4)_3 \cdot 16H_2O$	BDH
$ZnSO_4 \cdot 7H_2O$	BDH
$FeSO_4 \cdot 7H_2O$	BDH
$CuSO_4 \cdot 5H_2O$	BDH
Tris - Hcl	BDH
Nacl	BDH
NaOH	BDH
HCl	BDH
Ethanol	BDH
Acetone	BDH
Aniline blue	BDH
$CdSO_4$	BDH
$Al_2(SO_4)_3$	BDH
Formaldehyde	BDH
Acetic acid	BDH
Borax	Sigma Chemical Co.
Toluidine Blue	Sigma
L.R.White	The London Resin Company Ltd.
Sodium acetate	BDH
Glutaraldehyde	BDH

The image from pollen & pollen tubes were grabbed on a screen, pollen germination was counted and pollen tubes were measured using the dimension function on the microscale image analyser (MSIA). Functions of MSIA include:

(a). **Calibrate function:-** It provides operations that prepare the image for measurements, by setting calibration scales & by manually or automatically picking out the features of the interest to be analysed for instance, colour thresholding, setting scale, region.

(b). **Dimension function:-** Allows measurement of normal (straight) & poly lines (curves).

(c). **Scan objects function:-** Causes MSIA to search, detect & measure all discrete objects in the current threshold region of the interest.

2.5 Growth-room conditions

Plants were grown in a growth chamber at a constant temperature of 25°C under constant fluorescent illumination of 230 μ moles m^2s^{-1} light intensity, 271 x 10¹⁷ photon photosynthetically active radiation. conditions which induced flowering throughout the year. Seeds were planted in cocofibre compost in pots perforated from the bottom. The pots were placed in the plastic trays and watered as frequently as necessary. After flowering, flowers were used for these studies.

2.6 Glasshouse conditions

The glasshouse ambient temperature was 20°C. In order to provide sixteen hour day-length for the plants, high pressure 400 w sodium lamps, type SON/T (Anon, 1973) were suspended 1.5 m. above the bench

2.7 Formulating pollen germinating medium for *Arabidopsis thaliana*

2.7.1 Flower collection

Fresh, opened, mature flowers from different plants of the wild type genotype were collected. The pollen from fresh flowers was collected gently with a fine paint brush and mixed together to reduce flower to flower effects.

2.7.2 Media preparations

Twentyone media (Table-3.1) containing $Ca(NO_3)_2 \cdot 4H_2O$, H_3BO_3 , $MgSO_4 \cdot 7H_2O$ and KNO_3 at 6 concentrations (0, 100, 200, 300, 400 and 500 ppm) were prepared from preliminary observations it was revealed that 200 ppm of calcium nitrate gave good results, therefore from media M-7 to M-21, calcium nitrate was kept constant (200 ppm). From initial studies it was also observed that 12% (w/v) sucrose gave best results, therefore this sucrose concentration was used in all media. Studies of sucrose concentration were also made after developing standard medium (SM). The results are shown in table-3.4.

2.7.3 Sample preparations

Four replicates for each medium, making total of 84 samples were prepared. The fresh pollen collected was transferred to porous cellophane on top of a layer of a filter paper saturated with the germination medium in a Petri dish (fig. 2.1).

2.7.4 Incubation

Samples were incubated at 20°C for 24 h. In a separate preliminary experiment this temperature (20°C) was found to be optimum for pollen germination.

2.7.5 pH

The pH was maintained with Tris-HCl buffer at pH 7.5. This pH was found to be optimum for *Arabidopsis* pollen (Table-3.5, 3.7)

2.7.6 Data recording

In each sample 10 readings were recorded for pollen tube length (PTL) and 5 for pollen germination percentage (PG%). The pollen tubes were measured in micro meters (μm) and germination at the basis of percentage (%). Measurements were made randomly, with the help of a microscope equipped with a computer image analysis system.

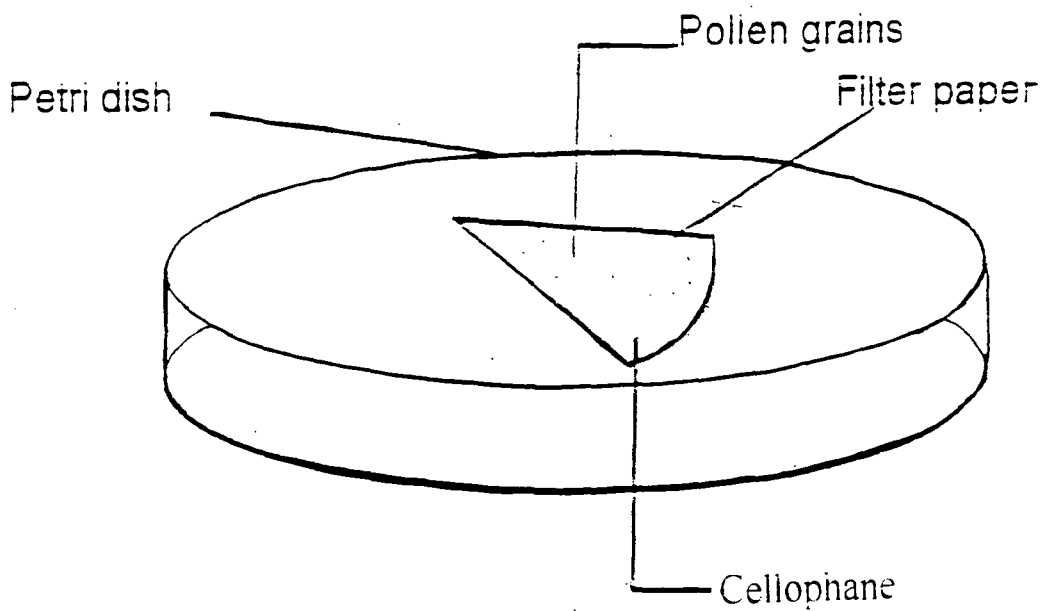


Fig. 2.1. Shows the experimental devices for the pollen germination of the *Arabidopsis thaliana* pollen under newly developed pollen germinating medium (Table-3.1).

2.7.7 Pollen germination assessment

A pollen grain was regarded as germinated if it showed a protuberance the length of which was at least equal to the diameter of grain (Cook and Walden, 1982). Grains which had the whole of their irregular mass of cytoplasm extruded from the pollen cell wall were defined as burst. Non-germinated pollen grains showed no sign of biological activities (Herrero and Johnson, 1980). Germination results were calculated according to the formula of Herrero and Johnson.

$$\text{Pollen germination (\%)} = \frac{\text{Pollen germinated}}{\text{Total counts} + \text{burst pollen grains}} \times 100$$

2.8 Effect of sucrose

To study the sucrose effects on pollen germination and growth of *Arabidopsis* pollen, 9 sucrose levels (0, 5, 10, 12, 15, 20, 25, 30, 35% w/v) were selected for study.

Four samples for each sucrose level were prepared as per the procedure already outlined. The samples were incubated at 20°C for 24 h and observations were recorded as described above.

2.9 Effect of metals on pollen germination and growth

To evaluate the effect of some heavy metals on the pollen germination and tube growth, 4 metallic salts including $Al_2(SO_4)_3 \cdot 16H_2O$, $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ were chosen for studies. With the exception of copper sulphate five concentration levels (10, 20, 30, 40, 50 ppm) were selected, while for copper

sulphate comparatively low levels (1, 2, 3, 4, 5 ppm) were taken. In preliminary studies copper was found to be lethal to pollen of *Arabidopsis* at above 10 ppm.

Four replicates of each medium, making total of 105 samples, with added metal levels in SM were prepared. The pollen was mixed from fresh flowers and germinated as described above. The pH was maintained by Tris-HCl at pH 7.5. The samples were incubated for 24 h. and observations were recorded as per the procedure previously described.

2.10 Effect of buffer (Tris-HCl) on pollen germination and growth

To study the effect of Tris on the pollen germination and growth, three concentrations of Tris (25, 50, 75 ppm) and five pH levels (7.0, 7.5, 8.0, 8.5, 9.0) were studied. The SM was buffered with the above selected concentrations, and for each concentration five pH levels were maintained by adding HCl.

Four samples/concentration/pH, making a total of 60, together with controls, were prepared. The pollen collected from the fresh flowers was transferred to the Petri dishes containing the particular media. Samples were incubated for 24 h and observations were recorded as described above.

2.11 *In vivo* and *in vitro* comparison of pollen germination

To study the pollen growth, plants with active, healthy buds were uprooted and replanted as an individual plants in small cups with moist cocofibre compost. The flowers were emasculated before opening and isolated from other populations by putting polythene bags over the cups containing the plants. The opened flowers on the plants were also removed prior to emasculation in order to avoid any chance of pollen transfer to the emasculated flowers. The flowers were examined under a

microscope over different intervals of time. After stigma maturation, emasculated flowers were hand pollinated and the time was noted. After a 3 h incubation period at 20°C the flowers were excised from mother plants, floral parts were removed carefully with needle and forceps and the gynaecial portions were retained in microcentrifuge tubes for staining.

2.12 Staining of ovaries

For staining of ovaries the following procedure was adopted.

In the first instance the ovaries were immersed in 70% ethanol solution for 10 minutes, then transferred to 4% (w/v) *NaOH* solution. The microcentrifuge tubes containing ovaries in *NaOH* were kept in a water bath at 60°C for 1 h to hydrolyse the ovaries. After hydrolysis the ovaries were transferred in phosphate buffer solution (0.05%, pH.7.0) for 5 minutes, then transferred on a glass slide and mounted in drop of decolourised aniline blue. Every specimen was squashed gently under a cover slip. The specimen were viewed under the fluorescence microscope, using a ultra-violet excitation filter. The measurement of pollen tubes running from stigmas in the ovaries were made manually with the eye piece scale fitted to the microscope.

For the in vitro studies samples were prepared in SM and incubated for 3h. The data was recorded as per the procedure outlined earlier.

2.13 Genotype responses towards pollen medium

To study the response of different genotypes towards the new recommended standard medium (SM) for *Arabidopsis* pollen, 10 lines, three from the Landsberg group including Wild, NW-45, NW-46 and seven from the Columbia group in-

cluding seven sub-populations of the single Columbia line, 18/60, 20/60, 21/60, 22/60, 23/60, 24/60 and 25/60. Four pollen samples for each genotype, making a total of 40, were prepared with SM. Samples were incubated for 24 h and pollen germination and growth data were recorded as per the procedure described earlier.

2.14 *In vitro* rate of pollen germination and growth

To evaluate the *in vitro* rate of pollen germination and growth of *Arabidopsis* pollen under different temperature regimes, 5 time intervals (3, 5, 10, 15, 24 h) and 8 temperatures (0, 5, 10, 15, 20, 25, 30, 35°C) were selected for studies.

4 samples/temp./time interval/ were prepared and incubated under particular temperatures for prescribed time intervals, and observations for pollen germination and pollen growth were recorded as described above.

2.15 Formulating the pollen germinating medium for *Cicer arietinum*

In order to single out a simple germinating medium for pollen of *Cicer arietinum* 30 pollen germinating media (Table-3.2) were evaluated for the purpose.

2.15.1 Plant material

Seeds of chick pea were planted in pots filled with cocofibre compost medium and raised to flowering in the glass house of the Botanical Garden of University of Durham , under a ambient temperature 20°C in May, 1991.

2.15.2 Flower collection

Fresh flowers which opened in the morning were excised at 10 am. The pollen of all flowers was mixed thoroughly to avoid flower-to-flower variation.

2.15.3 Sample preparation

After mixing the pollen collected from flowers, pollen grains were transferred onto the cellophane surface already moistened with the relevant media on filter paper in the Petri dishes.

2.16 Effect of temperature on pollen germination and growth in *A. thaliana*

Three genotypes of *Arabidopsis* (Landsberg group) including Wild, NW-45 and NW-46 were selected to study their pollen response towards varying temperatures. The plants were grown in one of the growth chambers of the Department of Biological Sciences, University of Durham. In the growth chamber plants were raised at a constant temperature of 25°C under constant fluorescent illumination conditions which induces flowering round the year. Plants were raised to flower in pots perforated from bottom containing cocofibre compost media. The pots were kept in trays and watered through the bottoms periodically.

2.16.1 Pollen collection

Fresh opened flowers were collected from the plants grown in growth chamber. The pollen from different flowers was pooled together to reduce the flower-to-flower variations.

2.16.2 Use of pollen germinating medium

The following new pollen germinating medium developed for *Arabidopsis* pollen (Table-3.1) was used for pollen germination and growth.

$H_3BO_3 = 100$ ppm

$Ca(NO_3)_2 \cdot 7H_2O = 200$ ppm

$MgSO_4 \cdot 7H_2O = 100\text{ppm}$

$KNO_3 = 100\text{ppm}$

Sucrose = 12

2.16.3 Effect of temperature

To assess the germination and growth of *Arabidopsis* pollen under various temperature regimes, 8 temp. levels (0, 5, 10, 15, 20, 25, 30, 35°C) were chosen. Temperature 20°C was treated as control.

2.16.4 Temperature and genotype interactions

To study the effect of temperature on genotypes, 3 lines of the Landsberg group, including Wild, NW-45, and NW-46, were selected and pollen was collected from their fresh flowers.

2.16.5 Sample preparation

Four samples /temp./genotype, making a total of 96, were prepared. The data were recorded as already described.

2.16.6 *In vivo* and *in vitro* comparisons under different temperatures

In order to study the response of pollen to temperature regime *in vivo* and *in vitro*, 5 temperature levels (10, 15, 20, 25, 30°C) and 3 genotypes (Wild, NW-45, NW-46) were selected. Flowers from plants grown in the growth chamber were collected and pollen was mixed with a fine paint brush.

Four samples /temp./genotype, making total a of 48, were prepared for *in vitro* germination and tube growth. The pollen was transferred on to a cellophane

surface on Whatman filter papers moistened with SM in Petri dishes. Samples were incubated for 24 h. pH was maintained with Tris-HCl (25 mM) at pH 7.5.

For *in vivo* studies the same procedure was followed as already mentioned in previous experiments.

2.16.7 Effect of temperature on pollen germination and growth in *Cicer arietinum*

To assess the effect of temperature on pollen of *C. arietinum*, 6 temperature levels (10, 15, 20, 25, 30, 35°C) were used for the studies. Fresh flowers were collected at 10 a.m, from the plants grown in the glass house of the Department of Biological Sciences. The pollen from flowers was mixed thoroughly. Pollen grains were transferred onto a cellophane surface on filter papers in 9 cm Petri dishes using the newly developed pollen germinating medium (H_3BO_3 , 1000 ppm, $Ca(NO_3)_2 \cdot 4H_2O$, 300 ppm, Sucrose, 8% w/v) for *C. arietinum*.

Four samples for each temperature were prepared and incubated for 1 hour at the required temperatures, and the observations were recorded as previously stated.

2.17 Impact of differential pollen growth rates on the sporophytic traits of *Arabidopsis thaliana*

Biological material

A. Recipient

Male-sterile (MS) line

B. Pollen donors

NW-28, NW-91, NW-46, Wild and CAD1

Cross combinations

MS x NW-28, MS x NW-91, MS x NW-46, MS x Wild, MS x CAD1

2.17.1 Raising of plants

The plants for the crossing of the pollen donors (PD) with male steriles were grown in the growth room, as described earlier. At the flowering stage the fresh flowers were excised from the donor parents and pollen was dusted on the stigmas of freshly opened male sterile flowers. For crossing, a single vigorous male-sterile plant was selected. Every PD was crossed with a separate raceme of the same chosen plant (Plate-2.1A). At maturity the seed was collected separately for each combination

2.17.2 Raising F1s

Two replications with a randomized complete block design, each in a separate perforated tray (22 x 34 cm), were planted. In order to water the experiment these trays were again placed in bigger trays. The experiment was planted in

Oct.,1992 and harvested in Jan. 1993. The seeds from each cross combination and parents were sown with 3 x 3 cm. row-row and plant-plant distances. In order to avoid possible nongerminability of any seeds, initially five seeds/hill were sown and thinned after one week by leaving one seedling/hill. The experimental trays after planting were kept under glasshouse conditions (Plate 2.1B), as already described.

2.17.3 Data recording

The data were recorded for the following characteristics.

1. Pollen tube growth rates (PTGRs)

To evaluate the pollen growth rates in different genotypes, the fresh flowers from plants of both replications were collected separately. Four pollen samples for each of the crosses and PDs, making a total of 80 for both replications were prepared in pollen germinating medium (**Baloch and Gates**, unpublished) using procedure already outlined. The samples were kept under 20°C temperature for five hours. The pollen tube growth was recorded.

Sporophyte growth characteristics

The following parameters were used:

- (a). **Main shoot/raceme initiation(MSI)**
- (b). **First flower initiation (FFI)**
- (c). **Days to bolting (DTB)**
- (d). **Rosette diameter (RD)**



Plate 2.1 (A) shows the crossing of the *Arabidopsis thaliana* male-sterile plant with pollen donors of different genotypes.
Plate 2.1 (B) shows the experimental growing conditions of F1s.

(a). Main shoot/raceme initiation:-

The days taken after planting (DAP) up to initiation of main shoot or raceme were recorded.

(b). First flower initiation:-

The days taken after planting up to emergence of first flower were recorded.

(c). Days to bolting:-

The days were recorded for the flowering after emergence of main raceme up to initiation of first flower.

(d). Rosette diameter:-

It has been observed that in *Arabidopsis* the rosette attains its full growth at the time of main shoot emergence. The diameter of rosette was measured at that time with a ruler.

2.18 Effect of salinity (*NaCl*) on the sporophyte and gametophyte of *A. thaliana*

Three experiments (Plates-2.2-2.3) were conducted to study some effects of salinity in pollen and plants of *A. thaliana*. The aims and details of experiments are described below.

The seeds for experiments were initially planted in autoclaved sterilised cocofibre compost in bottom-perforated plastic trays (22 x 34 cm) at 3 cm plant-to-plant and row-to-row distances, so as to encourage plants to grow vigorously. Initially five seeds/hill were planted to avoid possible non-germinability of any seed. The seedlings were thinned after one week by leaving one seedling/hill to grow. The plants were allowed to grow for one month and then some plants were transferred

to the MSS plant culture medium.

EXPERIMENT-1

2.18.1 Male-gametophyte growth: an index for prediction of salinity limits and selection for salt tolerance in plant species.

This experiment was conducted to address the following questions.

- (a). What are the effects of salinity on gametophytic characters, when pollen is collected from salt stress-free (SF) plants and tested in salt supplemented pollen germinating media, and when it is (pollen) collected from salt-stressed (SS) plants and is tested in salt stress-free pollen germinating media?
- (b). What are the effects of salinity on some agronomic traits of the sporophyte?
- (c). Is it possible to predict the salinity tolerance limits of a particular species by testing its pollen?
- (d). Does salinity have parallel effects on both gametophytic and sporophytic generations of a plant life cycle?

In this experiment (Plate-2.2a), two groups of plants were chosen for study. At flowering the one group of plants was transferred in MS solution without any salinity stress, another group was transferred to the salinity stress conditions by adding different levels of sodium chloride to MS solution.

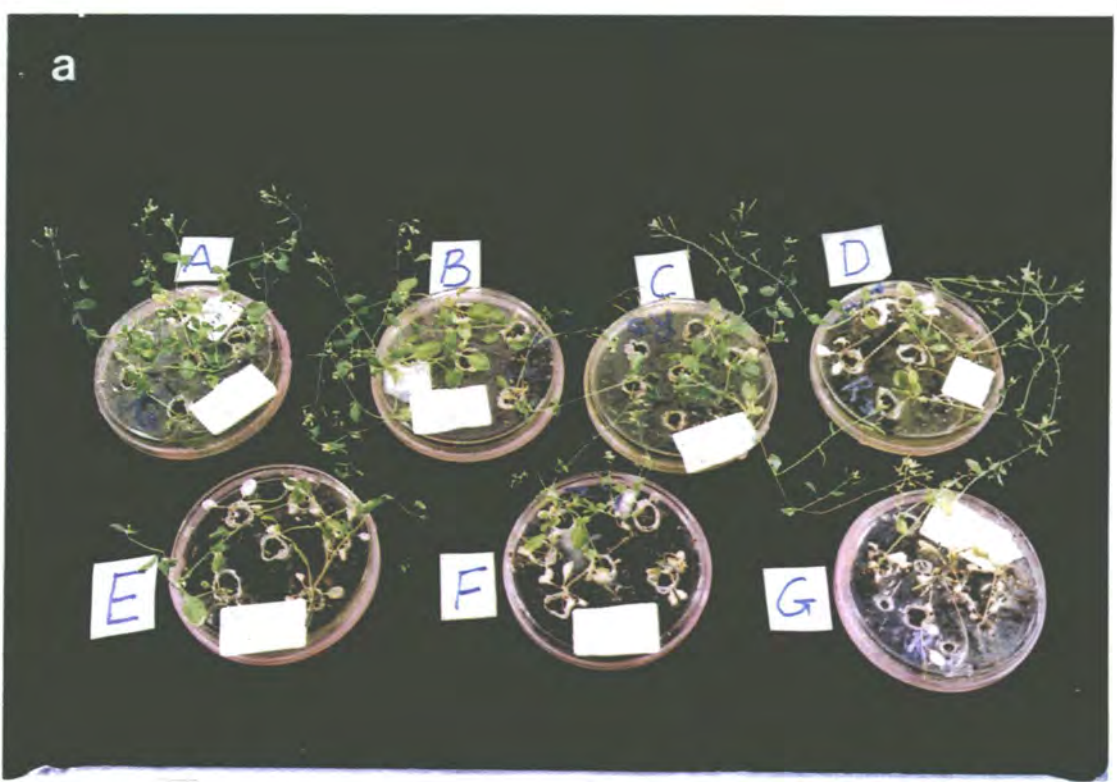


Plate 2.2 (a). Plants of *Arabidopsis thaliana* growing under different saline regimes (A = MS nutrient medium; B = 500 ppm; C = 1000 ppm; D = 2000 ppm; E = 3000 ppm; F = 4000 ppm; G = 5000 ppm).

Plate 2.2 (b). The wild type (WT) and male-sterile (MS) plants growing under different saline conditions (A = WT-HS; B = WT-SF; C = MS-SF; D = MS-HS). HS denotes high-stress (NaCl = 5000 ppm) and SF denotes stress-free.

For pollen studies, the pollen was first collected from the plants grown without stress. The pollen germination, pollen tube length and pollen growth rates data (Table-6.1) were developed by supplementing four *NaCl* concentrations (100, 500, 1000, 2000 ppm) in the **Baloch and Gates** (unpublished) *Arabidopsis* pollen germinating medium. The results showed that 2000 ppm was generally lethal for pollen germination and pollen tube growth. It was therefore decided to supplement 25% (500 ppm), 50% (1000 ppm), 100% (2000 ppm), 125% (3000 ppm), 150% (4000 ppm) and 175% (5000 ppm) of the semi-lethal dose for pollen to the plants. The intention was to observe how salinity affects the pollen through the intact plant system. At flowering the pollen of stressed plants was studied in pollen germinating medium without added salinity stresses and data was recorded.

EXPERIMENT-2

2.18.2 Fruit-setting, seed-setting, seed-filling and single & double-stress effects in response to the high salinity conditions

Single-stress (SS):-This term hereafter will be used for the pollen collected from stress-free plants and germinated in salt-added pollen germinating media.

Double-stress (DS):- This term will be used for pollen collected from salinity-stressed plants and germinated in salt-added pollen germinating media.

The experiment was conducted with the following aims:

- (a). To evaluate the impacts of **single-stress** and **double-stress** salinity regimes on the pollen germination and growth.
- (b). To investigate the effects of salinity on the fruit-setting, seed-setting and seed-filling.

2.18.2.1 Fruit-setting

Fruit-setting was calculated as the percent ratio of number of crosses attempted and number of successful crosses obtained.

$$\text{Fruit-setting (\%)} = \frac{\text{Attempted crosses}}{\text{Successful crosses}} \times 100$$

2.18.2.2 Seed production

The seed production was scored as the number of seeds per pod from successful crosses.

2.18.2.3 Filled seeds

Observations of the seeds of *Arabidopsis* revealed that its pod contains the following five types of seed:

- (A). Filled brown seeds (viable)
- (B). Filled black seeds (viable)
- (C). Filled blackish brown seeds (viable)
- (D). Shrivelled irregular black seeds (unviable)
- (E). Shrivelled irregular brown seeds (unviable)
- (F). Brownish broken seeds (unviable)

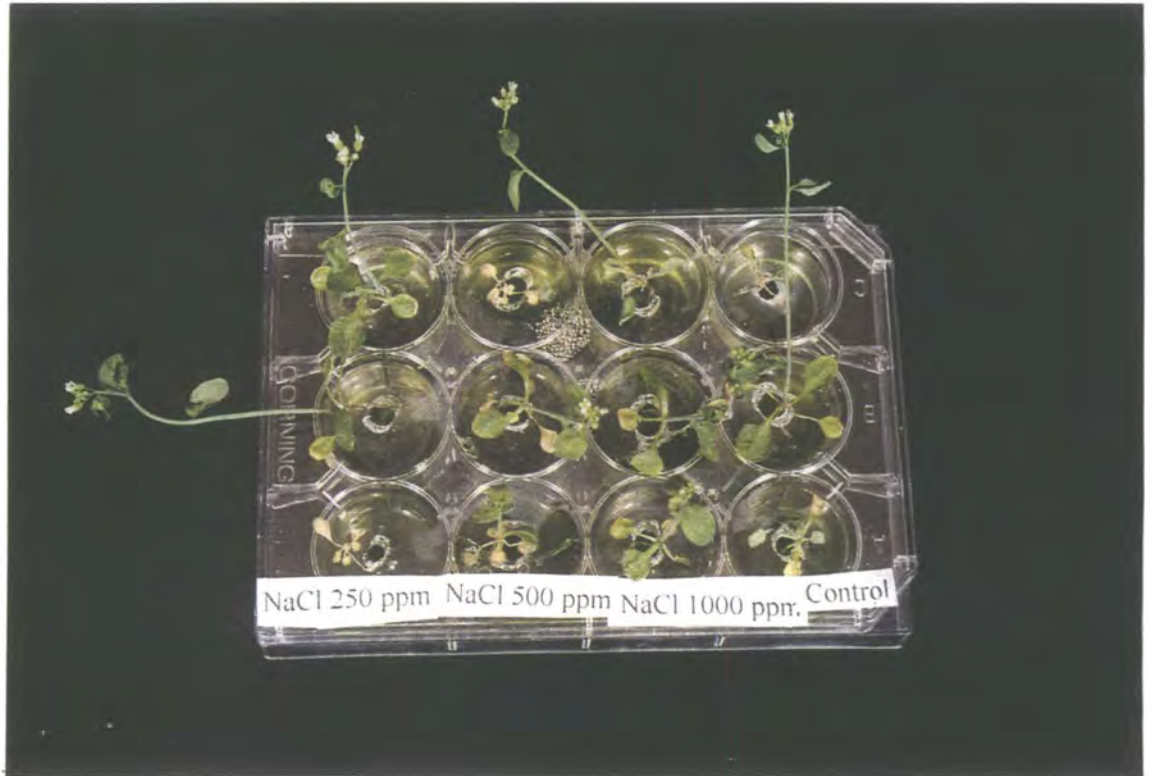


Plate 2.3 Seedlings of *Arabidopsis thaliana* raised with and without NaCl-stress regimes for observing the anatomical changes in the stem, leaf and root.

While testing *Arabidopsis* seed samples for seed viability, the seed germination tests showed that the seed-types, A, B, and C produced more than 97% seed germination, while none of the seeds for seed-types D, E and F germinated. Therefore the term "**Filled-seed**" means the seeds produced resembling seed-types A, B & C.

The experiment was conducted in the glasshouse of the Botanical Garden. The experiment was conducted with wild type and male-sterile (MS) line. The wild and male-sterile plants were raised to flowering in the same tray under identical growing conditions. At flowering four plants, two wild type and two male-sterile, were selected. Out of these four plants, two plants (one wild, one male-sterile) were separately transferred to the plant culture medium (MSS) in stress-free conditions without added salt. The remaining two plants were subjected to high salt stress (HS) conditions (5000 ppm) provided in the plant culture medium. These single plants were transferred in the pots filled with vermiculite (Plate-2.2b). The pots containing plants were in turn kept in slightly larger containers in the required culture medium. The solution was replaced after every four days with fresh medium.

After transplanting, the flowers that appeared in the first week were removed from plants in order to increase the duration of the saline environment on flower development. After one week the hybridization was started and the following possible crosses were performed:

MS-HS x wild-HS

MS-HS x wild-SF

MS-SF x wild-HS

MS-SF x wild-SF

After crossing the successful crosses were harvested and data was recorded.

EXPERIMENT-3

2.18.3 Anatomical changes brought by salinity in stem, leaf

and root of *Arabidopsis thaliana*

The seeds were planted in the compost and after eight days of their growth the small seedlings were transferred into the hydroponic half MSS plant culture medium. Initially plants were tried under 12 different *NaCl* levels ranging from 250 to 10000 ppm. It was observed that, being small seedlings, the plants could survived only up to the 1000 ppm. although in our previous experiments on salinity the plants tolerated the salinity doses up to the 5000 ppm. However plants in those experiments were transferred in latter stages.

After testing the nature of survival of plants, three concentrations (250, 500, 1000 ppm) were proposed to lay out the experiment. Three plants were transferred to each of the stress conditions and control (Plate-2.3). The plants were allowed to grow for 15 days under specified conditions.

After 15 days the plants were excised in three parts, stem, leaf and root. The stem samples were taken 1 cm above the rosette from each plant. The leaves of approximately equal size were chosen from each plant.

To carry out the light microscopy following procedure of Feder and Brien (1968) adopted by Serrato et al. (1991) was followed for fixing, embedding and staining the material:

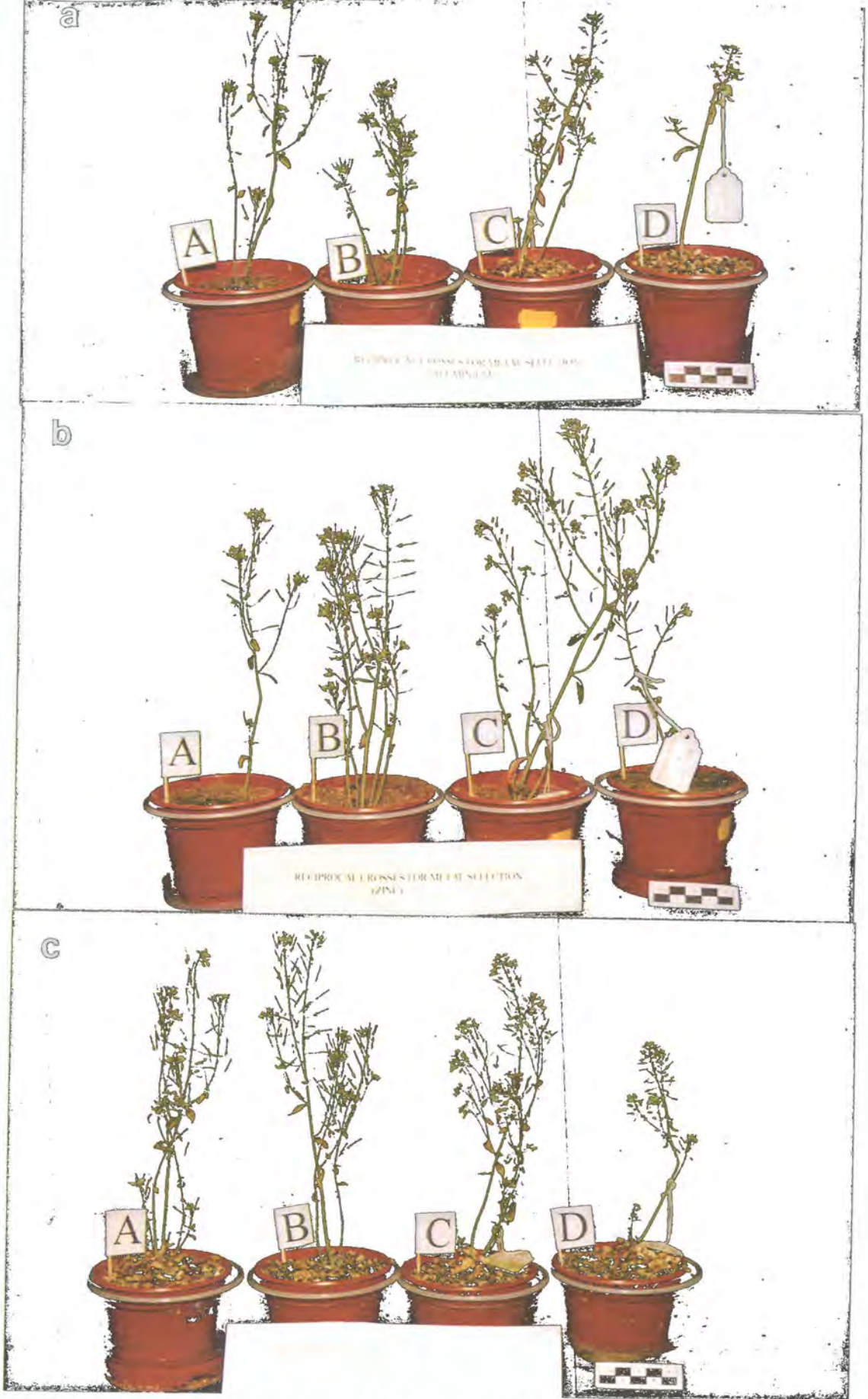


Plate 2.4 a-c. Plants of *Arabidopsis thaliana* raised for the reciprocal crosses in presence and absence of heavy metal concentrations. Photographs a, b and c show the plants respectively for aluminium, zinc and copper stresses. The labels in the pots show A = WT-HS, B = WT-SF, C = MS-SF and D = MS-HS (where WT denotes wild type; HS denotes high stress level of particular metal and SF denotes stress-free).

The segments of stem, leaves and roots were fixed for 24 h in FAA (formaldehyde 40%, acetic acid glacial, ethanol 100% 1:1:3 ratio). Dehydration took place in ethanol series, 12.5, 25, 50, 75, 95% 1 h and 100% twice for 1 h. The samples were kept in 50% dry alcohol and L.R.white over night. After dehydration the material was embeded in resin by incubating them for 24 h at 60°C.

One micron sections were managed on Dupont instrument, Sroval, MT2-B Ultra Microtome. The sections were stained with Toluidine Blue O (TBO) 0.05% in acetate buffer 0.1 M (sodium acetate 0.2 M, acetic acid 0.2 M) at pH 4.4 for 1 min. The sections were examined under bright field microscopy and were photographed with microscope fitted camera using 400 Fujicolor film.

2.19 Fruit-setting, seed-setting and seed-filling in *A. thaliana* in response to high metal concentrations.

In order to study the consequences of metals on fruit-setting, seed-setting and seed-filling in *Arabidopsis thaliana*, four metals comprising of $CuSO_4$ (20 ppm), $Al_2(SO_4)_3$ (500 ppm), $ZnSO_4$ (300 ppm) and $CdSO_4$ (200 ppm), were chosen with their specific concentrations depending upon their toxicity to *Arabidopsis* pollen. This experiment was based on results obtained from the experiment whose results are described in chapter-3.

The aim of the experiment was to gain insight into variation in fruit-setting, seed-setting and seed-filling in *Arabidopsis* when:

1. The maternal plant is raised under a high-stress (HS) metal regime and pollen is donated from a plant under high-stress (HS) or stress-free (SF) conditions.
2. The maternal plant is grown in stress-free conditions and similar pollen donors are used.

Raising of plants

The plants were raised to flower in glasshouse conditions as already described in this chapter. At flowering the plants were similarly transferred to stressed and stress-free conditions (Plate-2.4) and crosses were made as described for salinity exp.-2 (chapter-6). For the three metals (copper sulphate, aluminium sulphate and zinc sulphate) wild type and male-sterile line were used. For cadmium sulphate experiment the genotype CAD1, which is cadmium sensitive, was used with the same male-sterile line. The following possible reciprocal crosses for cadmium were made:

MS-HS x CAD1-HS

MS-HS x CAD1-SF

MS-SF x CAD1-HS

MS-SF x CAD1-SF

After ripening of the crossed siliquas the data for fruit-setting, seed-setting and seed-filling was collected and analysed.

2.20 Anatomical changes brought about by heavy metals in stem, leaf and root of *Arabidopsis thaliana*

Plants were grown in compost for 15 days under similar conditions in growth room as already described in previous experiments, except that in the case of genotype CAD1 (cadmium-sensitive), the plants were grown up to 30 days because CAD1 is late to initiate bolting.

The seedlings were transferred in 12-well plastic boxes (Plates 8.1, 8.18) provided by Baby Sterline Limited Hampshire (UK). The plants were subjected to the selective concentrations of $CdSO_4$ (0, 100, 150, 200 ppm), $Al_2(SO_4)_3$ (0, 200, 300, 500 ppm), $ZnSO_4$ (0, 100, 200, 300 ppm) and $CuSO_4$ (0, 10, 15, 20 ppm). The plants were allowed to grow up to 5 days under particular stress conditions. In the case of the cadmium-sensitive line the plants could not survive beyond 5 days, hence these were only left to grow for 2 days.

2.20.1 Preparing material for light microscopy (LM)

(a). **Stem**:- The 1 cm portions of stem was selected immediately above the rosette. The selected portion was sub-divided in 1 mm pieces.

(b). **Leaf**:- The 2nd node leaf was selected for studies which emerged in plants after putting them in stress conditions.

(c). **Root**:- The 1 cm portions of young lateral roots were selected for studies. tried to select some younger portions of lateral roots developed under stress After selecting the required material, the assay adopted by Vazquez et al. (1992) for fixation and embedding the material for LM was followed with slight changes. The details of the procedure are as under:

2.20.1.1 Fixation

The material was fixed in 2% glutaraldehyde containing 0.1% Na_2S in 0.1M phosphate buffer (pH 7.2), for 24 hours.

2.20.1.2 Dehydration

Samples were dehydrated in an acetone series as follows:

Acetone 12.5%, 25.5%, 50% and 75% (one hour); 100% (one hour) twice. 50% acetone, 50% L R White (over night). L R White 100% for 2 days, changing the solution morning and evening.

After 2 days the specimen were transferred in microcentrifuge tubes filled with L R White resin. The samples were incubated over night in oven at 65°C. After embedding the material, sectioning was done similarly with a microtome as described above. The sections were metachromatically stained with Touildine blue 0.5%, examined under microscope and photographed as already stated.

2.21 Computing facilities and data analysis

The text and tabulations were prepared on the **SUN & HP work-stations** by using the **TeX-word processing** package. Computing facilities used were provided by Northumbrian University Multiple Access Computer (NUMAC), at the University of Durham. The analysis of varience (ANOVA) of data were performed by using **Minitab soft-ware** package through the **Novel system (Gresley server)** in Computer Centre University of Durham. The graphics were produced using **Cricket graphics soft-ware version 1.3.1** package in **Word for Windows, version 3.1** in Department of Biological Sciences.

Chapter III

Formulating pollen germinating medium

Despite the general suitability of Brewbaker and Kwack (1963) medium for a wide range of species with binucleate pollen, their study showed that plants with trinucleated pollen in the *Cruciferae* and *Compositae* showed relatively poor pollen germination (Leduce et al., 1990). Although *Arabidopsis* pollen is trinucleate, a simple pollen germinating medium was developed for its pollen germination and tube growth.

Twentyone media (Table-3.1) were tested to identify a satisfactory pollen germinating medium for the pollen of *Arabidopsis*. Highly significant ($P \leq 0.001$) differences were obtained among the media tested for pollen germination as well as for pollen tube length (Table-3.3).

3.0.1 Effect on pollen germination (PG)

Results indicate that for pollen germination percentage (PG%) highly significant differences ($f = 183.15$, $df = 20$, $P \leq 0.001$) were obtained among the pollen germinating media. The highest pollen germination was recorded (Table-3.3, Fig. 3.1) in medium M-3 (80.01%). The least PG% was given by M-21 (0.95%). Overall M-3 gave 38.92% (M-2) to 98.81% (M-21) more pollen germination than the rest of the media tested.

3.0.2 Effect on pollen tube growth

Highly significant interactions ($f = 84.36$, $df = 20$, $P \leq 0.001$) were recorded

in respect with pollen tube length among the tested media. The longest pollen tube length (Table-3.3, Fig. 3.2) was produced by pollen germinating medium M-3 (750.65 μm). The range of increase of longest pollen growing medium M-3 varied from 33.82% (M-2) to 93.24% (M-21) over all the media tested.

3.1 Effect of sucrose

To study the effect of sucrose on the pollen of *Arabidopsis*, nine sucrose levels (0, 5, 10, 12, 15, 20, 25, 30, 35% w/v) were selected for the purpose. Sucrose levels showed highly significant differences for pollen germination ($f = 226.40$, $df = 8$, $p \geq 0.001$) and for pollen tube length ($f = 149.57$, $df = 8$, $p \leq 0.001$)

Highest PG% (Table-3.4, Fig. 3.3) was obtained at 12% (81.77%) closely followed by 15% (79.39%). The low ranking concentrations were 0% (5.20%) and 35% (20.04%) which produced 93.94% and 75.49% less germination than the best sucrose (12%) level. The remaining sucrose levels produced a decline in germination, from 32.05% (25%) to 41.51% (5%) in relation to 12% (w/v) of sucrose.

Sucrose levels also significantly affected pollen tube length (PTL). The highest (Table-3.4, Fig. 3.4) pollen tube length was produced by the 12% (821.45 μm) level, closely followed by 10% (789.98 μm), 5% (786.34 μm) and 15% (694.56 μm). The shortest tubes were produced by the 35% (65.23 μm) followed by 0% (113.29 μm). Other sucrose levels yielded 45.60% (20%) to 49.05% (30%) shorter pollen tubes in comparison to the best suited sucrose level (12% w/v).

3.2 Effect of buffer (Tris-HCl) and pH

Tris-HCl was selected as a buffer for inclusion as a buffering agent in the pollen germinating medium of *Arabidopsis* pollen. Initially four (25, 50, 75, 100

mM) concentrations were selected. But its high inhibition gave negligible pollen germination and growth at 100 mM, hence data for 100 mM has not been included.

3.2.1 Tris-HCl

Highly significant ($p \leq 0.005$) differences were observed among the levels of Tris-HCl for pollen germination and pollen tube length. The results (Table-3.10, Fig.3.9) indicate that at every pH level the Tris-HCl concentrations affected pollen germination and pollen tube length variably. The pollen germination of 47.58% (pH 9.0) to 76.49% (pH 7.5) was obtained in case of 25 mM, the pollen germination of 19.88% (pH 8.5) to 33.41% (pH 7.5) was recorded at 50mM and from non-germination of pollen to 1.52% was noted in case of 75mM added concentration of Tris-HCl in the pollen germinating medium.

Similarly highest pollen tube length (Table-3.12, Fig. 3.11) ranging from 534.72 μm to 729.80 μm were recorded with 25 mM of Tris-HCl. The PTL was adversely affected in case of 50 mM (0%-156.03 μm) and 75 mM (0%-39.23 μm).

3.2.2 pH

While assessing pH effects, non significant ($p \leq .05$) differences were obtained among pH. Tables, 3.5, 3.7 (Figs. 3.6, 3.8) indicate that differences among pH range for pollen germination and pollen tube growth were minimal under differing conditions.

3.3 *In vivo* and *in vitro* comparisons

For comparative studies *in vivo* and *in vitro* in respective to pollen tube growth in *Arabidopsis*, highly significant ($P \leq 0.001$) differences were noted. The tubes

travelled faster *in vivo* than *in vitro*. The *in vivo* increase growth percentage over *in vitro* (Table-3.9, Fig. 3.9) varied with genotypes differently, i.e. 78.78%, 81.35%, and 83.49% for NW-45, wild type and NW-46 respectively.

3.4 Genotype responses towards newly developed pollen germinating medium

In order to observe the responses of genotypes of *Arabidopsis* towards the newly developed pollen germinating medium, 10 genotypes were studied. They gave significant differences, for PG ($f = 2.39$, $df = 9$, $p \leq 0.05$) and PTL ($f = 23.54$, $df = 9$, $p \leq 0.001$). The highest pollen germination (Table-3.10, Fig. 3.10) was displayed by genotype NW-45 (82.10%). The other genotypes produced less pollen germination, ranging from 3.12% (wild type) to 21.69% (20/60). In respect of PTL, highly significant ($p \leq 0.005$) differences were found among genotypes. The longest pollen tube (Fig. 3.15) was produced by genotype 25/60 (684.51 μm), the shorter by NW-46 (520.71 μm). The percent increase (Table-3.10) of the longest pollen tube growing genotype (26/60), over all other genotypes ranged from 4.86% (23/60) to 23.92% (NW-46).

3.5 Formulating a simple germinating medium for *Cicer arietinum*

Thirty media (Table-3.2) were selected to determine a pollen germinating medium for pollen of *Cicer arietinum*. Highly significant responses for pollen germination ($f = 36.46$, $df = 29$, $p \leq 0.001$) and pollen tube length ($f = 18.63$, $df = 29$, $p \leq 0.001$) were noted towards different levels of mineral elements and sucrose percentages.

The highest pollen germination (Table-3.11, Fig. 3.12) was given by medium M-8 (59.05%). The range of increase of M-8 medium over other media varied from

26.21% (M-12) to 92.09% (M-6).

The longest pollen tubes (Table-3.11, Fig. 3.13) were recorded in medium, M-9 (351.70 μm). The range of increase of M-9 medium over other media varied from 29.22% (M-13) to 89.19% (M-4).

Table-3.1: Pollen germinating media tested for germination and growth of *Arabidopsis* pollen with different levels of calcium nitrate, boric-acid, magnesium sulphate, potassium nitrate and 12% (w/v) sucrose.

Media	Calcium nitrate	Boric acid	Magnesium sulphate	Potassium nitrate	Sucrose% (w/v)
M-1	0 ppm	100 ppm	100 ppm	100 ppm	12%
M-2	100 ppm	100 ppm	100 ppm	100 ppm	12%
M-3	200 ppm	100 ppm	100 ppm	100 ppm	12%
M-4	300 ppm	100 ppm	100 ppm	100 ppm	12%
M-5	400 ppm	100 ppm	100 ppm	100 ppm	12%
M-6	500 ppm	100 ppm	100 ppm	100 ppm	12%
M-7	200 ppm	0 ppm	100 ppm	100 ppm	12%
M-8	200 ppm	200 ppm	100 ppm	100 ppm	12%
M-9	200 ppm	300 ppm	100 ppm	100 ppm	12%
M-10	200 ppm	400 ppm	100 ppm	100 ppm	12%
M-11	200 ppm	500 ppm	100 ppm	100 ppm	12%
M-12	200 ppm	100 ppm	0 ppm	100 ppm	12%
M-13	200 ppm	100 ppm	200 ppm	100 ppm	12%
M-14	200 ppm	100 ppm	300 ppm	100 ppm	12%
M-15	200 ppm	100 ppm	400 ppm	100 ppm	12%
M-16	200 ppm	100 ppm	500 ppm	100 ppm	12%
M-17	200 ppm	100 ppm	100 ppm	0 ppm	12%
M-18	200 ppm	100 ppm	100 ppm	200 ppm	12%
M-19	200 ppm	100 ppm	100 ppm	300 ppm	12%
M-20	200 ppm	100 ppm	100 ppm	400 ppm	12%
M-21	200 ppm	100 ppm	100 ppm	500 ppm	12%

Bold line shows the selected pollen germinating medium (M-3)

Table-3.2: Pollen germinating media tested for germination and growth of *Cicer arietinum* pollen containing different concentrations of boric- acid, sucrose and a constant level of calcium nitrate.

Media	Boric acid	Calcium nitrate	Sucrose% (w/v)
M-1	500 ppm	300 ppm	10%
M-2	500 ppm	300 ppm	8%
M-3	500 ppm	300 ppm	6%
M-4	500 ppm	300 ppm	4%
M-5	500 ppm	300 ppm	2%
M-6	500 ppm	300 ppm	1%
M-7	1000 ppm	300 ppm	10%
M-8	1000 ppm	300 ppm	8%
M-9	1000 ppm	300 ppm	6%
M-10	1000 ppm	300 ppm	4%
M-11	1000 ppm	300 ppm	2%
M-12	1000 ppm	300 ppm	1%
M-13	2000 ppm	300 ppm	10%
M-14	2000 ppm	300 ppm	8%
M-15	2000 ppm	300 ppm	6%

(Table 3.2 continued):- Pollen germinating media tested for germination and growth of *Cicer arietinum* pollen containing different concentrations of boric acid, sucrose and a constant level of calcium nitrate.

M-16	2000 ppm	300 ppm	4%
M-17	2000 ppm	300 ppm	2%
M-18	2000 ppm	300 ppm	1%
M-19	3000 ppm	300 ppm	10%
M-20	3000 ppm	300 ppm	8%
M-21	3000 ppm	300 ppm	6%
M-22	3000 ppm	300 ppm	4%
M-23	3000 ppm	300 ppm	2%
M-24	3000 ppm	300 ppm	1%
M-25	4000 ppm	300 ppm	10%
M-26	4000 ppm	300 ppm	8%
M-27	4000 ppm	300 ppm	6%
M-28	4000 ppm	300 ppm	4%
M-29	4000 ppm	300 ppm	2%
M-30	4000 ppm	300 ppm	1%

Table-3.3: Effect of different levels of calcium nitrate, boric acid, magnesium sulphate, and potassium nitrate on the pollen germination percentage and tube growth (μm) in *Arabidopsis thaliana* (wild type).

Media	Pollen germination (%)	%decrease v/s better medium	Pollen tube length (μm)	%decrease v/s better medium
M-1	44.38 \pm 3.67	-44.53	415.41 \pm 14.54	-44.76
M-2	48.87 \pm 2.46	-38.92	496.74 \pm 45.61	-33.82
M-3	80.01 \pm 5.13	-	750.65 \pm 29.98	-
M-4	47.67 \pm 2.85	-40.41	616.63 \pm 24.87	-17.85
M-5	26.60 \pm 4.70	-66.75	361.55 \pm 87.53	-51.83
M-6	8.20 \pm 0.27	-89.75	158.05 \pm 21.56	-78.94
M-7	47.35 \pm 3.74	-40.82	442.24 \pm 60.71	-41.08
M-8	42.76 \pm 5.22	-46.56	352.10 \pm 66.46	-53.09
M-9	40.34 \pm 3.25	-49.58	208.02 \pm 42.77	-72.28
M-10	38.33 \pm 1.49	-52.09	146.04 \pm 26.69	-73.88

(Table 3.3 continued):- Effect of different levels of calcium nitrate, boric acid, magnesium sulphate, and potassium nitrate on the pollen germination percentage and tube growth (μm) in *Arabidopsis thaliana* (wild type).

M-11	36.44 \pm 1.20	-54.45	185.00 \pm 23.54	-75.35
M-12	8.04 \pm 1.39	-89.95	117.54 \pm 22.67	-84.34
M-13	41.30 \pm 3.15	-48.38	279.87 \pm 66.69	-62.72
M-14	19.98 \pm 19.98	-75.03	148.45 \pm 24.19	-80.22
M-15	15.64 \pm 2.02	-80.45	122.29 \pm 25.86	-83.71
M-16	5.92 \pm 2.20	-92.60	76.83 \pm 14.94	-89.76
M-17	6.49 \pm 0.91	-91.89	154.74 \pm 22.57	-79.38
M-18	33.99 \pm 5.36	-57.52	360.79 \pm 73.63	-51.94
M-19	4.76 \pm 1.29	-94.05	153.41 \pm 10.18	-79.56
M-20	2.25 \pm 0.64	-97.19	90.50 \pm 11.82	-87.94
M-21	0.95 \pm 0.42	-98.81	50.73 \pm 8.74	-93.24
F (df)	183.15 (20)	-	84.36 (20)	-
P \leq	0.001	-	0.001	-

Table-3.4: Effect of different levels of sucrose on pollen germination and growth in *Arabidopsis thaliana* (wild type).

Sucrose% (w/v)	Pollen germination (%)	% decrease v/s better level	pollen tube length (μm)	% decrease v/s better level
	***		***	
0	5.20 \pm 1.62	-93.64	113.29 \pm 19.23	-86.21
5	47.82 \pm 4.04	-41.51	786.34 \pm 73.64	-4.27
10	53.37 \pm 3.68	-34.73	789.98 \pm 56.15	-3.83
12	81.77 \pm 2.44	-	821.45 \pm 67.52	-
15	79.39 \pm 3.89	-2.91	694.56 \pm 116.60	-15.45
20	53.11 \pm 4.61	-35.05	446.86 \pm 4.97	-45.60
25	55.56 \pm 3.69	-32.05	440.95 \pm 8.91	-46.32
30	52.22 \pm 2.46	-36.13	418.49 \pm 14.19	-49.05
35	20.04 \pm 2.32	-75.49	65.23 \pm 4.64	-92.06
F (df)	226.40 (8)	-	149.57 (8)	-
P \leq	0.001	-	0.001	-

*** = Significant P \leq 0.001

Table-3.5: Effect of pH levels on the pollen germination (%) of *Arabidopsis thaliana* (wild type).

pH levels	Tris-HCl 25mM	Tris-HCl 50mM	Tris-HCl 75mM
	ns	ns	ns
pH-7.0	61.16±2.01	21.92±0.70	nd
pH-7.5	76.49±2.42	33.41±3.05	1.52±0.54
pH-8.0	68.11±5.46	25.17±3.13	1.31±0.50
pH-8.5	56.06±1.78	19.88±4.48	nd
pH-9.0	47.58±6.32	nd	nd

ns= Non significant $P \leq 0.05$

nd= Means not determined

Table-3.6: Effect of Tris-HCl concentrations on pollen germination (%) of *Arabidopsis thaliana* (wild type).

Tris-HCl	pH-7.0	pH-7.5	pH-8.0	pH-8.5	pH-9.0
	***	***	***	***	***
25mM	61.16±2.01	76.49±2.42	68.11±5.46	56.06±1.76	47.58±6.32
50mM	21.92±0.70	33.41±3.05	25.17±3.13	19.88±4.48	nd
75mM	nd	1.52±0.54	1.31±0.50	nd	nd

***= Significant $P \leq 0.005$

nd= Means not determined

Table-3.7: Effect of pH levels on the pollen growth (μm) of *Arabidopsis thaliana* (wild type).

pH levels	Tris-Hcl 25mM	Tris-Hcl 50mM	Tris-Hcl 75mM
pH-7.0	ns 553.14±18.01	ns 98.88±17.85	ns nd
pH-7.5	729.80±12.19	156.03±3.66	40.60±2.19
pH-8.0	680.35±16.88	60.54±6.16	39.23±2.34
pH-8.5	647.77±41.13	41.91±3.88	nd
pH-9.0	534.72±47.33	nd	nd

ns= Non significant $P \leq 0.05$

nd= Means not determined

Table-3.8: Effect of Tris-HCl levels on the pollen growth (μm) of *Arabidopsis thaliana* (wild type).

Tris-HCl level	pH-7.0	pH-7.5	pH-8.0	pH-8.5	pH-9.0
	***	***	***	***	***
25mM	553.14±18.01	729.80±12.29	680.35±16.88	647.77±41.13	534.72±47.33
50mM	98.88±17.85	156.03±3.66	60.54±6.16	41.91±3.88	nd
75mM	nd	40.60±2.19	39.23±2.34	nd	nd

***= Significant $P \leq 0.005$

nd= Means not determined

Table-3.9: *In vivo* and *in vitro* comparison of pollen tube growth among 3 genotypes of *Arabidopsis*, incubated for 3 hours.

Genotype	<i>in vitro</i> pollen tube length (μm)	<i>in vivo</i> pollen tube length (μm)	% decrease of <i>in vitro</i> over <i>in vivo</i>
	***	***	
NW-45	150.65 \pm 15.56	710.0 \pm 99.24	78.78
NW-46	109.12 \pm 10.24	661.24 \pm 91.18	83.49
Wild type	122.46 \pm 13.59	656.90 \pm 140.11	81.35

***= Significant $P \leq 0.001$

Table-3.10: Genotype response towards a new medium for pollen germination and tube growth in *Arabidopsis thaliana*.

Genotype	Pollen germination (%)	% decrease v/s better genotype	Pollen tube length (μm)	% decrease v/s better genotype
Wild type	79.54 \pm 11.07	-3.12	578.80 \pm 29.58	-15.44
NW-45	82.10 \pm 4.09	-	647.76 \pm 38.45	-5.37
NW-46	67.54 \pm 6.71	-17.73	520.71 \pm 34.14	-23.92
18/60	72.60 \pm 8.35	-11.57	584.52 \pm 24.39	-14.61
20/60	64.72 \pm 5.55	-21.69	533.88 \pm 25.51	-22.00
21/60	72.19 \pm 7.11	-12.07	570.35 \pm 23.10	-16.68
22/60	64.52 \pm 3.74	-21.41	579.27 \pm 24.98	-15.37
23/60	73.56 \pm 9.91	-10.40	651.22 \pm 41.73	-4.86
24/60	72.74 \pm 6.90	-11.40	649.54 \pm 28.71	-5.11
25/60	75.57 \pm 6.20	-7.95	684.51 \pm 25.22	-
F (df)	2.39 (9)	-	23.54 (9)	-
P \leq	0.05	-	0.001	-

Table-3.11:Effect of different levels of boric acid and sucrose with fixed levels of calcium nitrate (ppm) on pollen germination and growth in *Cicer arietinum*

Media	Pollen germination (%)	% decrease v/s better medium	Pollen tube length (μm)	% decrease v/s better medium
	***		***	
M-1	18.35±5.84	-68.92	205.32±16.35	-41.62
M-2	15.06±6.25	-74.50	246.21±22.71	-29.99
M-3	20.89±3.11	-64.62	210.07±17.19	-40.27
M-4	8.83±1.40	-85.04	38.00±12.99	-89.19
M-5	6.72±2.11	-88.62	44.82±5.42	-87.26
M-6	4.67±0.82	-92.09	47.94±6.02	-86.37
M-7	37.40±4.66	-36.66	184.72±23.31	-47.48
M-8	59.05±4.72	-	175.79±29.34	-50.00
M-9	30.61±2.62	-48.16	351.70±29.85	-
M-10	13.76±1.57	-76.69	185.08±73.32	-47.37
M-11	19.84±5.68	-66.40	182.12±60.41	-48.22
M-12	43.57±3.72	-26.21	172.94±45.21	-50.83
M-13	29.63±8.94	-49.82	248.90±63.31	-29.22
M-14	21.85±8.44	-62.99	179.63±32.58	-48.92
M-15	16.07±7.10	-72.78	192.24±38.03	-45.34

Table-3.11 continued:Effect of different levels of boric acid and sucrose with fixed levels of calcium nitrate (ppm) on pollen germination and growth in *Cicer arietinum*

M-16	13.97±5.43	-76.34	163.13±10.21	-53.61
M-17	7.50±1.23	-87.30	100.48±24.66	-71.43
M-18	8.31±0.50	-85.93	63.87±9.55	-81.84
M-19	8.00±1.58	-86.45	161.79±85.17	-53.99
M-20	12.84±3.65	-78.25	189.94±87.20	-45.99
M-21	16.06±5.19	-72.80	171.66±34.97	-51.19
M-22	12.65±2.19	-78.57	85.51±61.05	-75.68
M-23	11.81±5.00	-80.00	71.52±24.22	-79.66
M-24	8.00±0.55	-86.45	59.04±7.48	-83.21
M-25	12.1±1.75	-79.50	109.46±11.29	-68.88
M-26	9.05±2.02	-84.67	99.94±16.46	-71.58
M-27	7.52±1.55	-87.26	75.38±19.01	-78.56
M-28	8.58±0.67	-85.00	38.70±10.40	-88.99
M-29	8.71±3.00	-85.24	49.44±4.34	-85.94
M-30	5.66±1.54	-90.41	39.16±8.65	-88.86
F (df)	36.46 (29)	-	18.63 (29)	-
P ≤	0.001	-	0.001	-

***= Significant $P \leq 0.005$

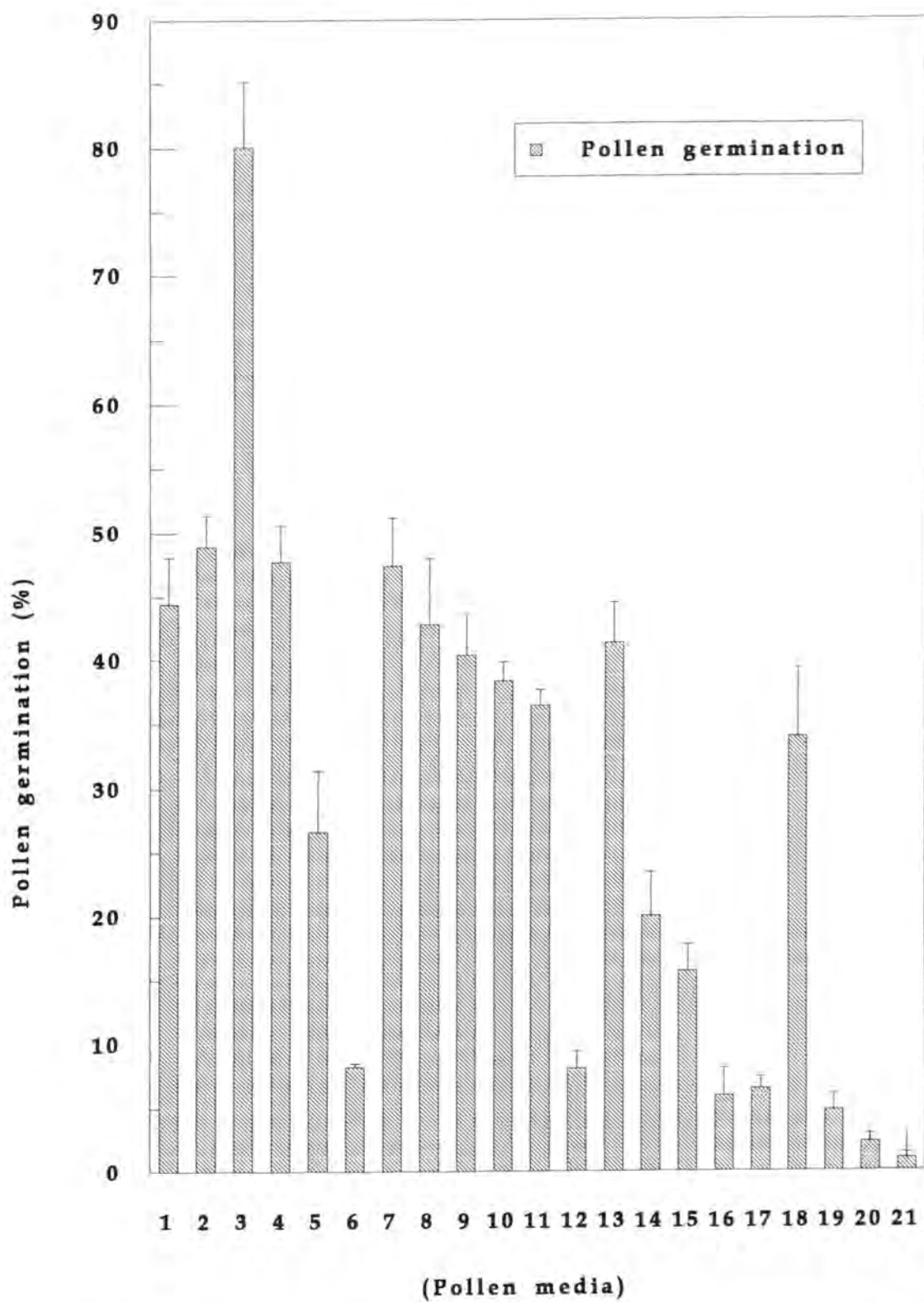


Fig. 3.1. Effect of different pollen germinating media on the pollen germination of *Arabidopsis thaliana* (wild type).

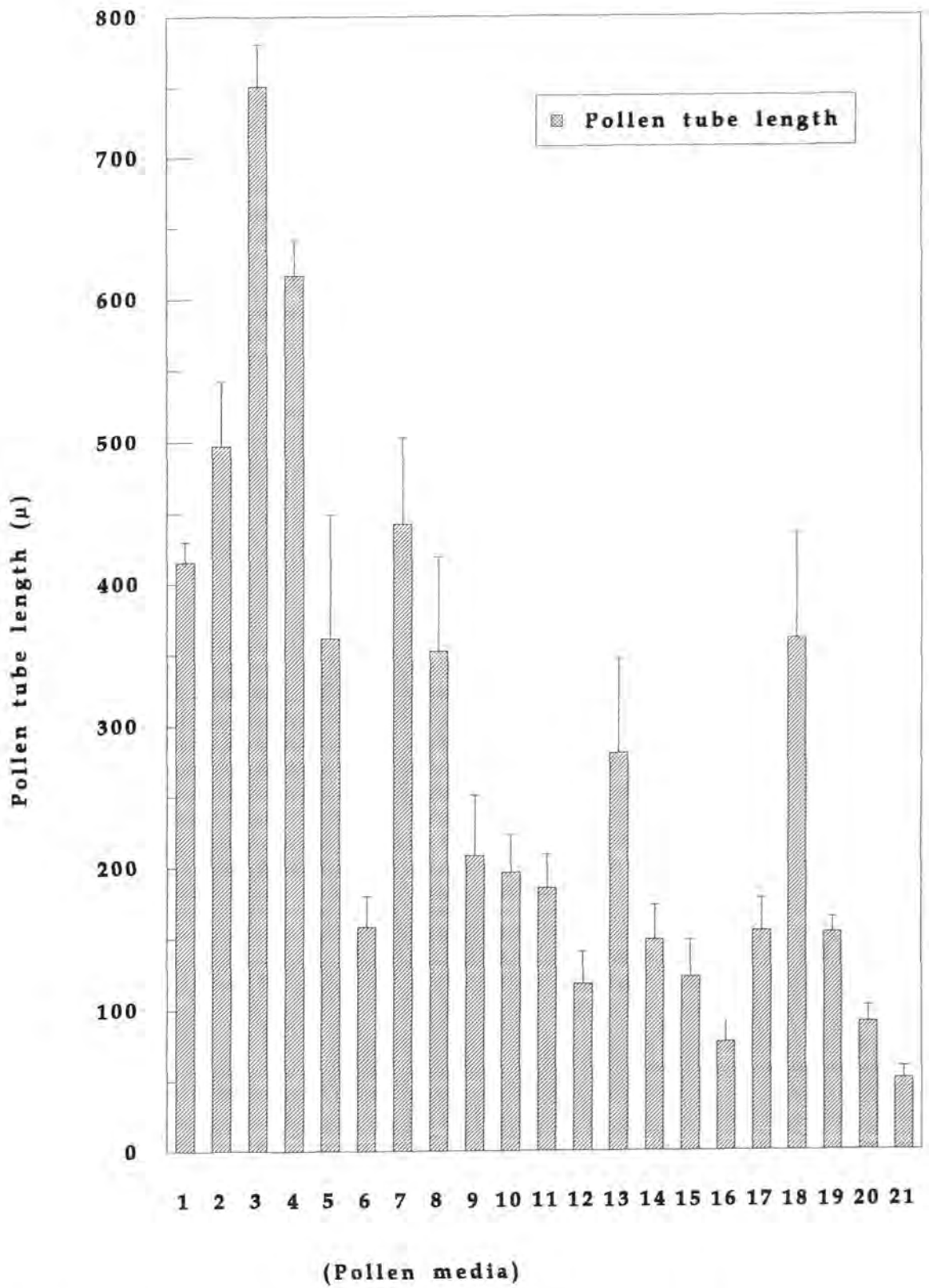


Fig. 3.2. Effect of different pollen germinating media on the pollen tube length of *Arabidopsis thaliana* (wild type).

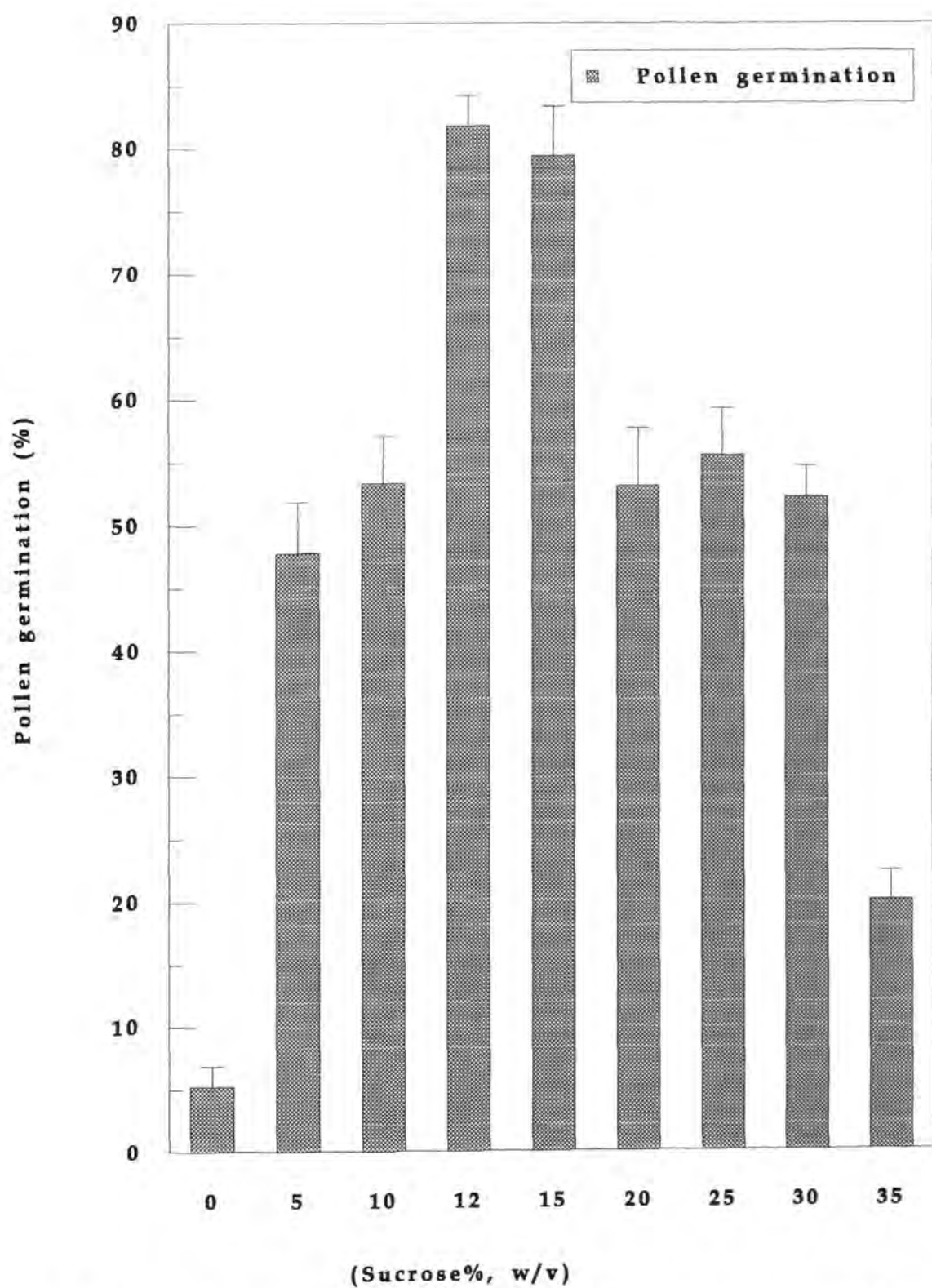


Fig.3.3. Effect of sucrose concentrations (w/v) on pollen germination of *Arabidopsis thaliana* (wild type).

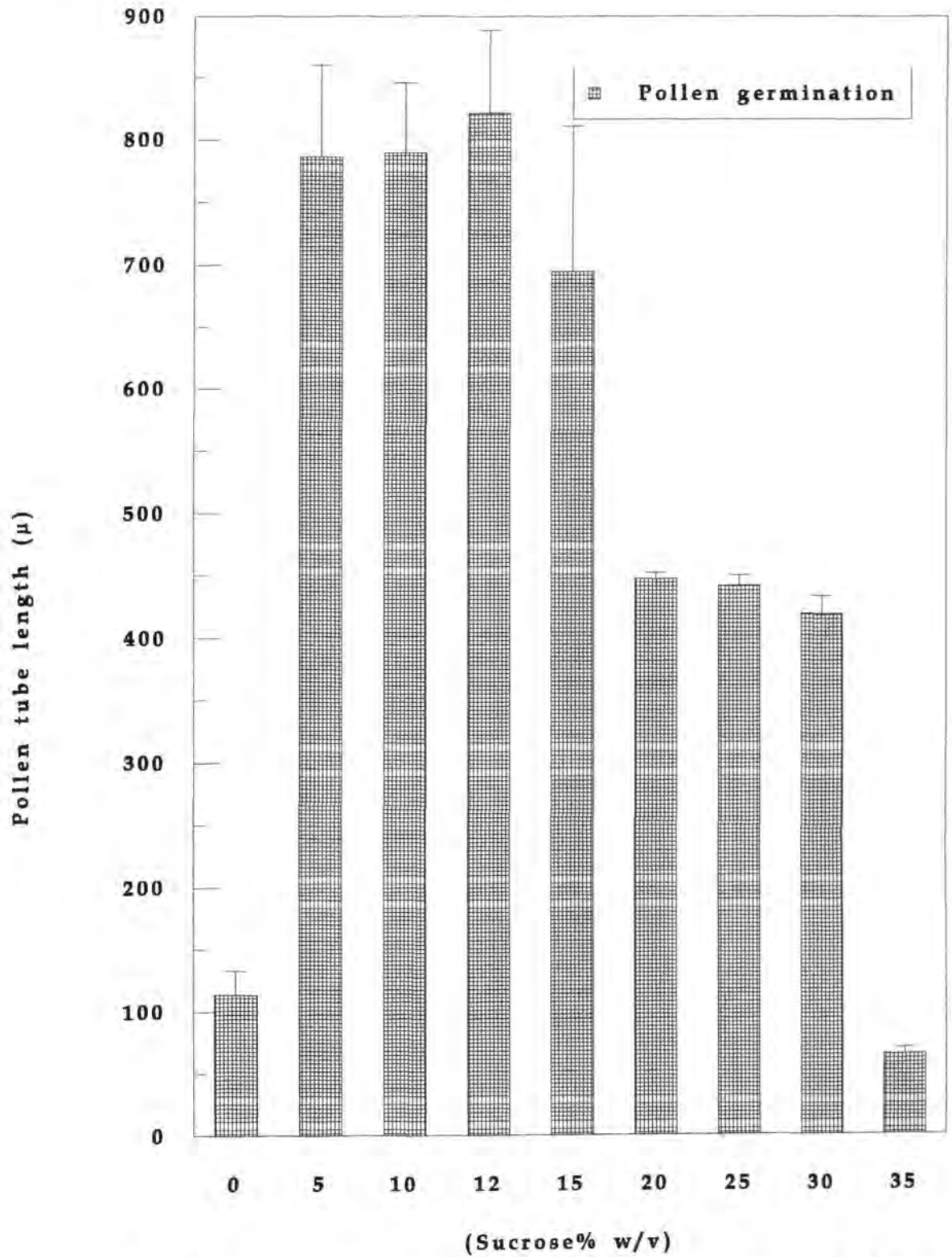


Fig.3.4. Effect of different levels of sucrose (w/v) on the pollen tube length of *Arabidopsis thaliana* (wild type).

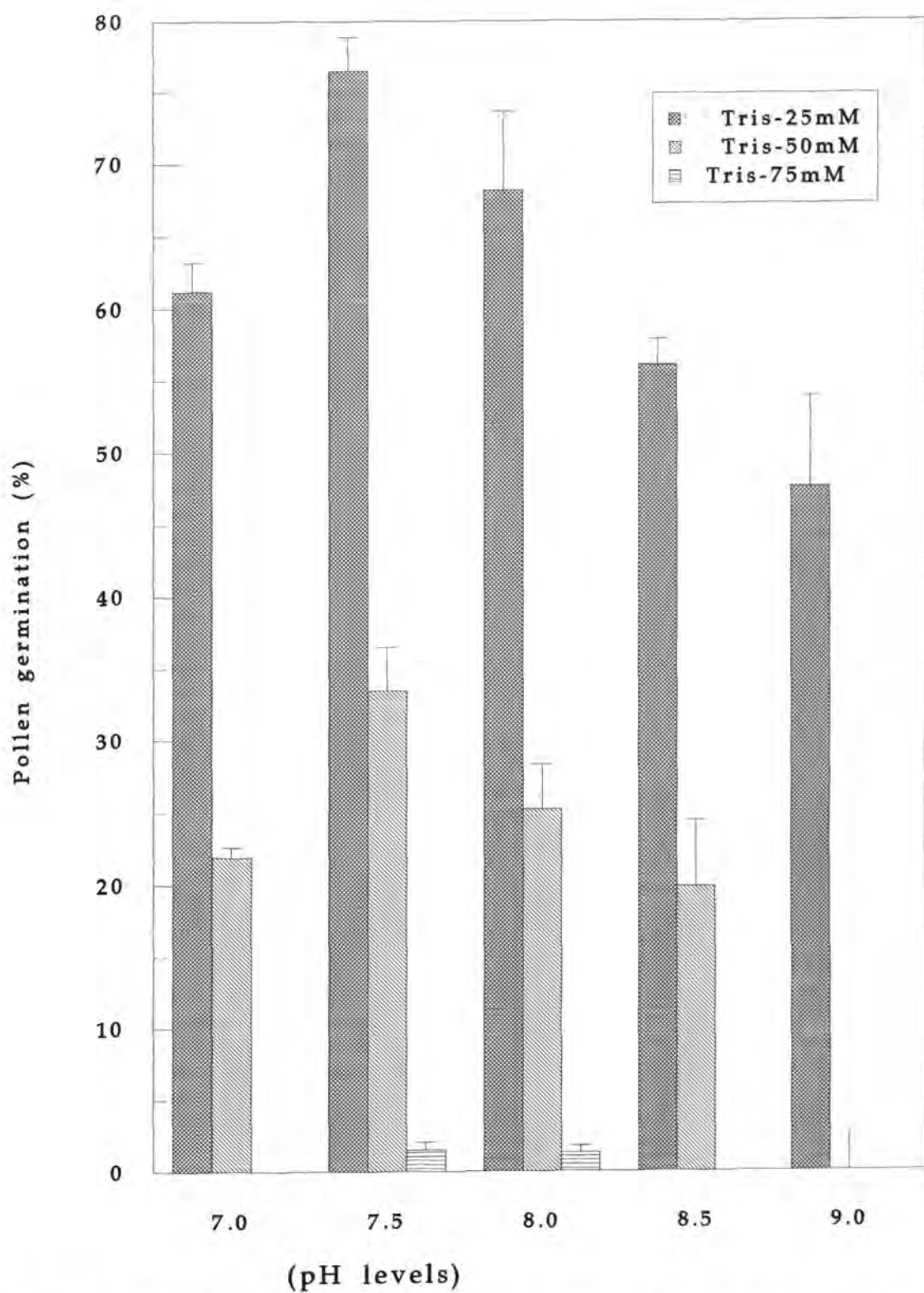


Fig. 3.5. Effect of pH levels on the pollen germination of *Arabidopsis thaliana* (wild type).

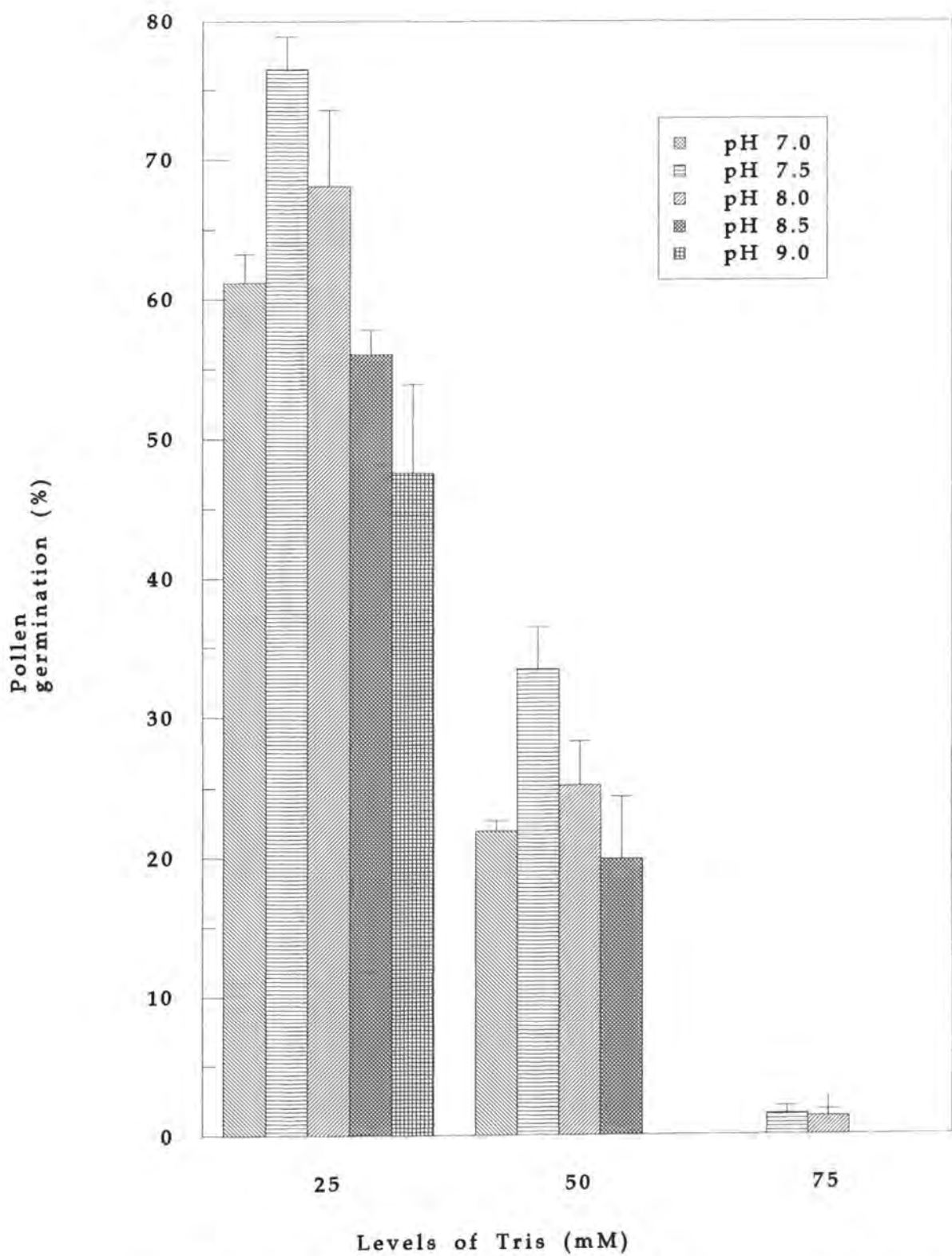


Fig.-3.6 . Effect of different levels of Tris-HCl on pollen germination of *Arabidopsis thaliana* (wild type).

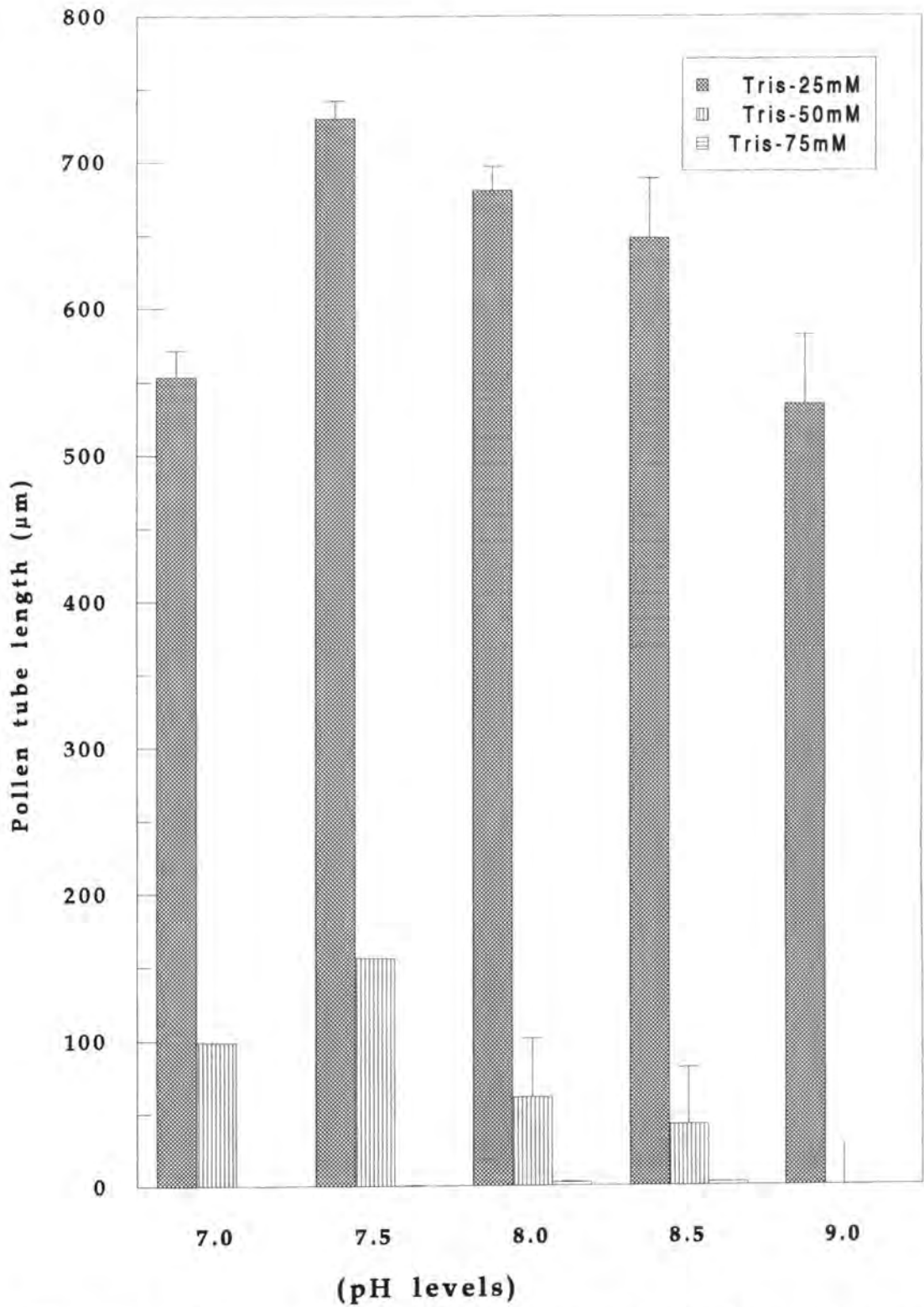


Fig. 3.7. Effect of different pH levels on the pollen tube length of *Arabidopsis thaliana* (wild type).

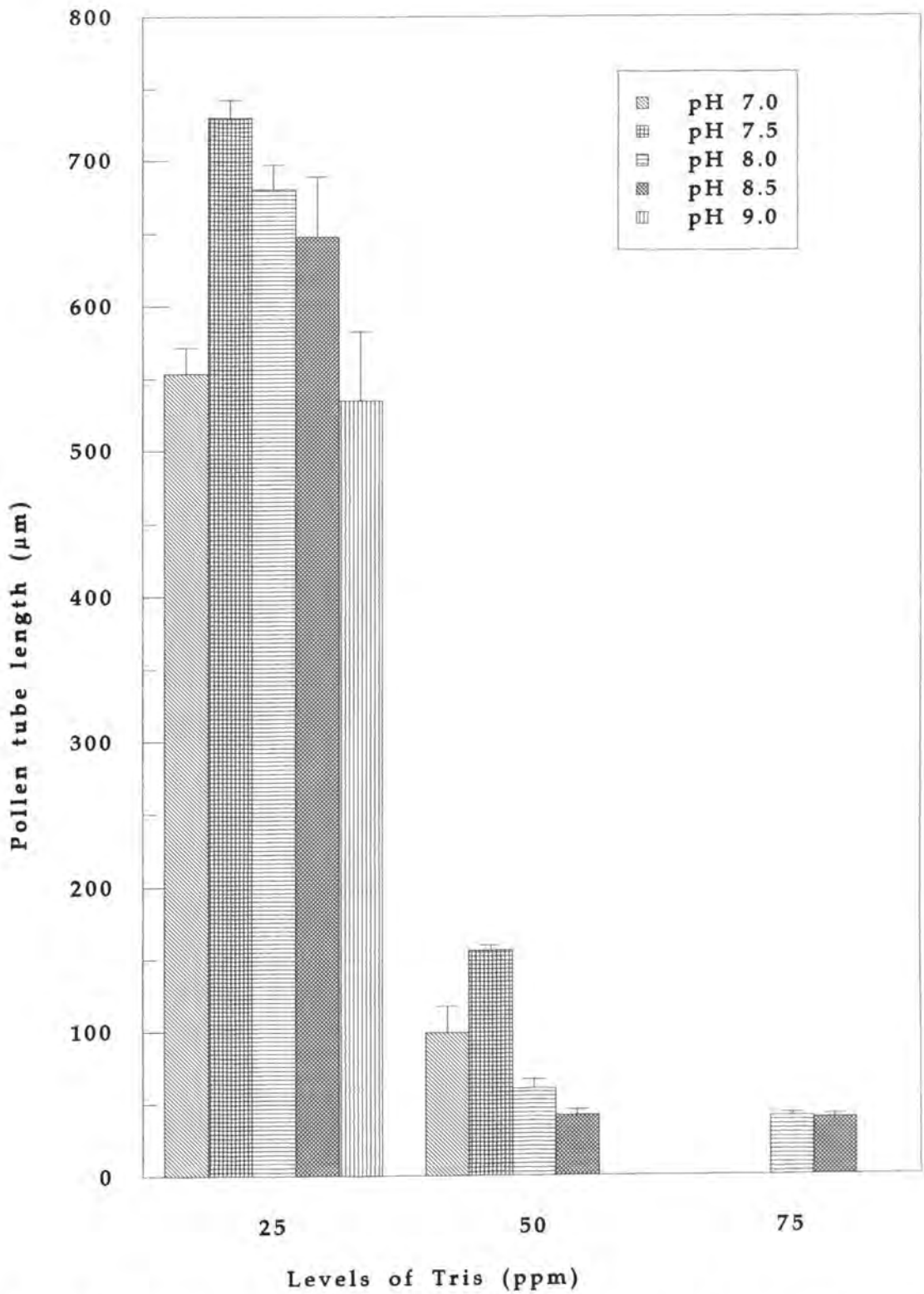


Fig. 3.8. Effect of different levels of Tris-HCl on the pollen tube length of *Arabidopsis thaliana* (wild type).

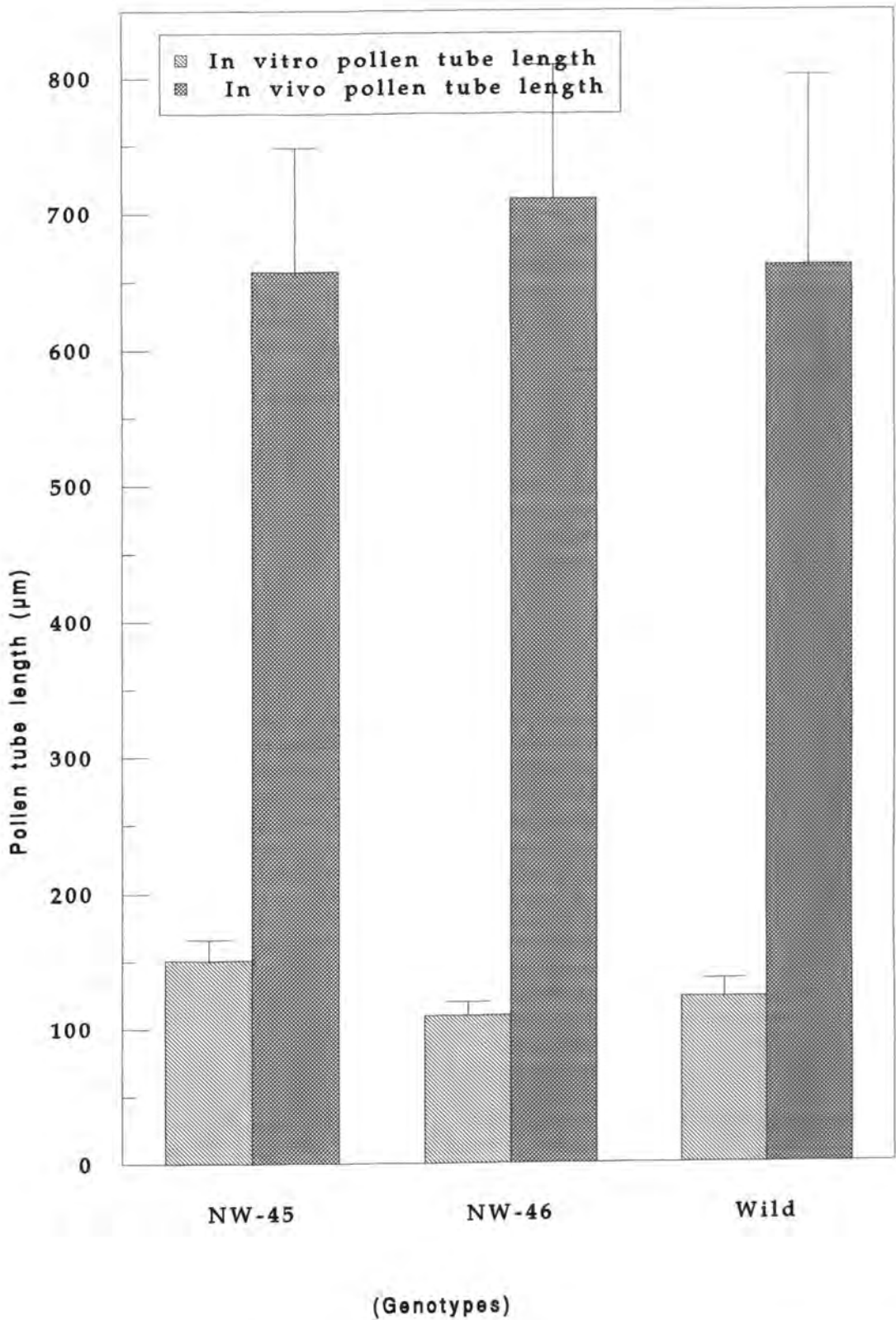


Fig. 3.9. In vitro and in vivo comparison of pollen tube length among three genotypes of *Arabidopsis thaliana* (wild type), after 3 hours incubation and pollination respectively.

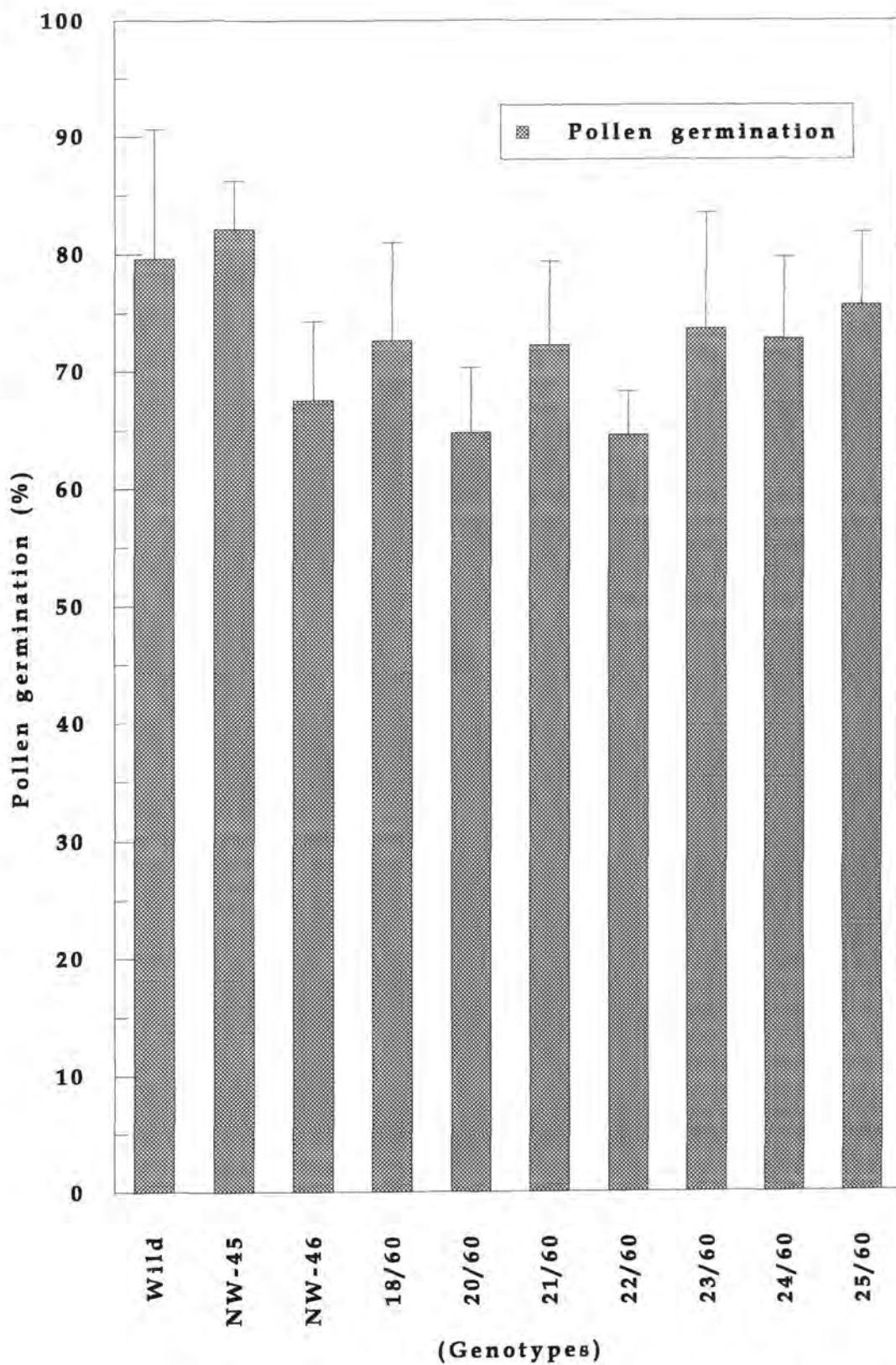


Fig. 3.10. Genotype response towards the medium developed for the pollen germination of *A. thaliana*

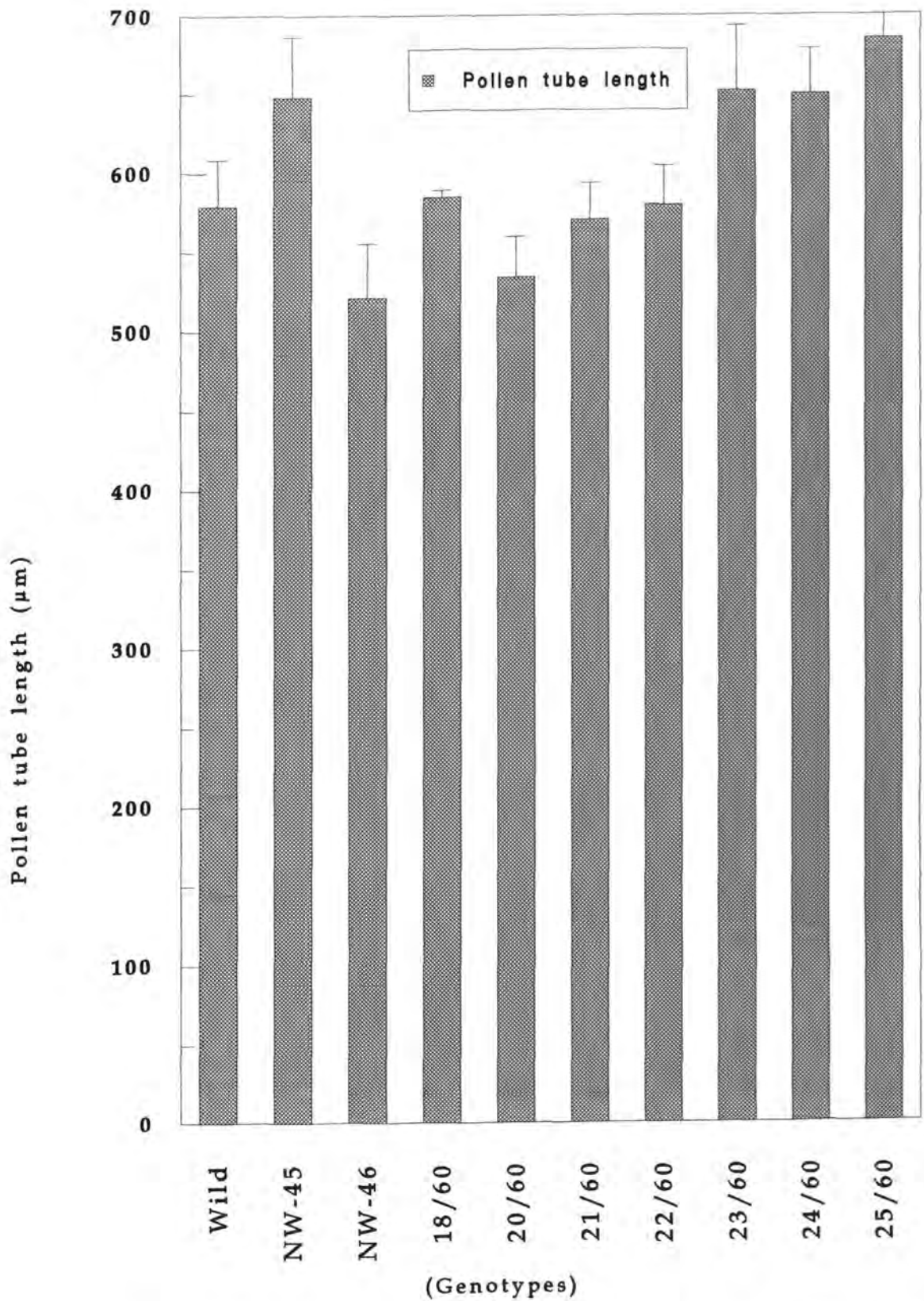


Fig. 3.11. Genotype response in respect of pollen tube length towards germinating medium developed for *Arabidopsis thaliana*

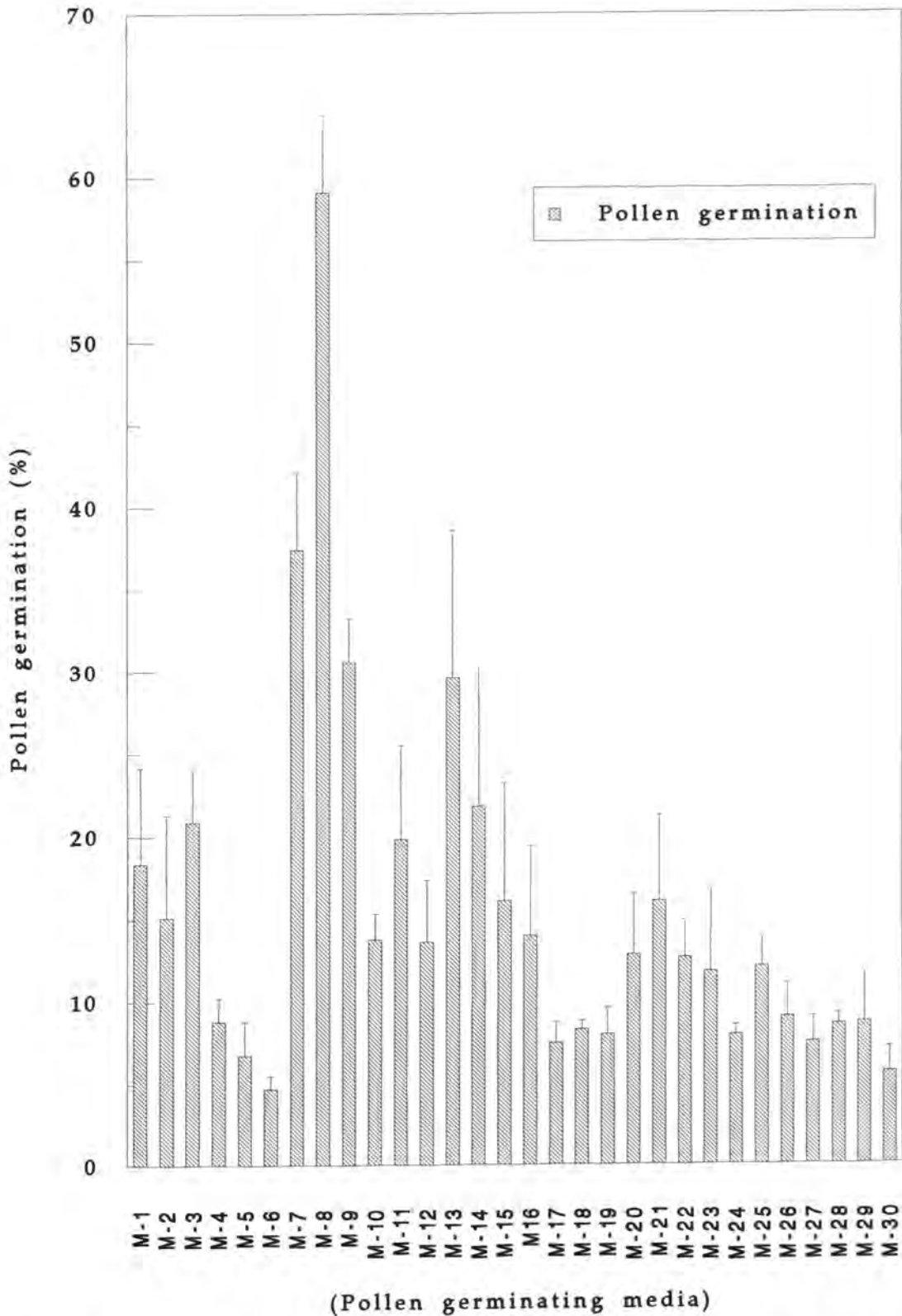
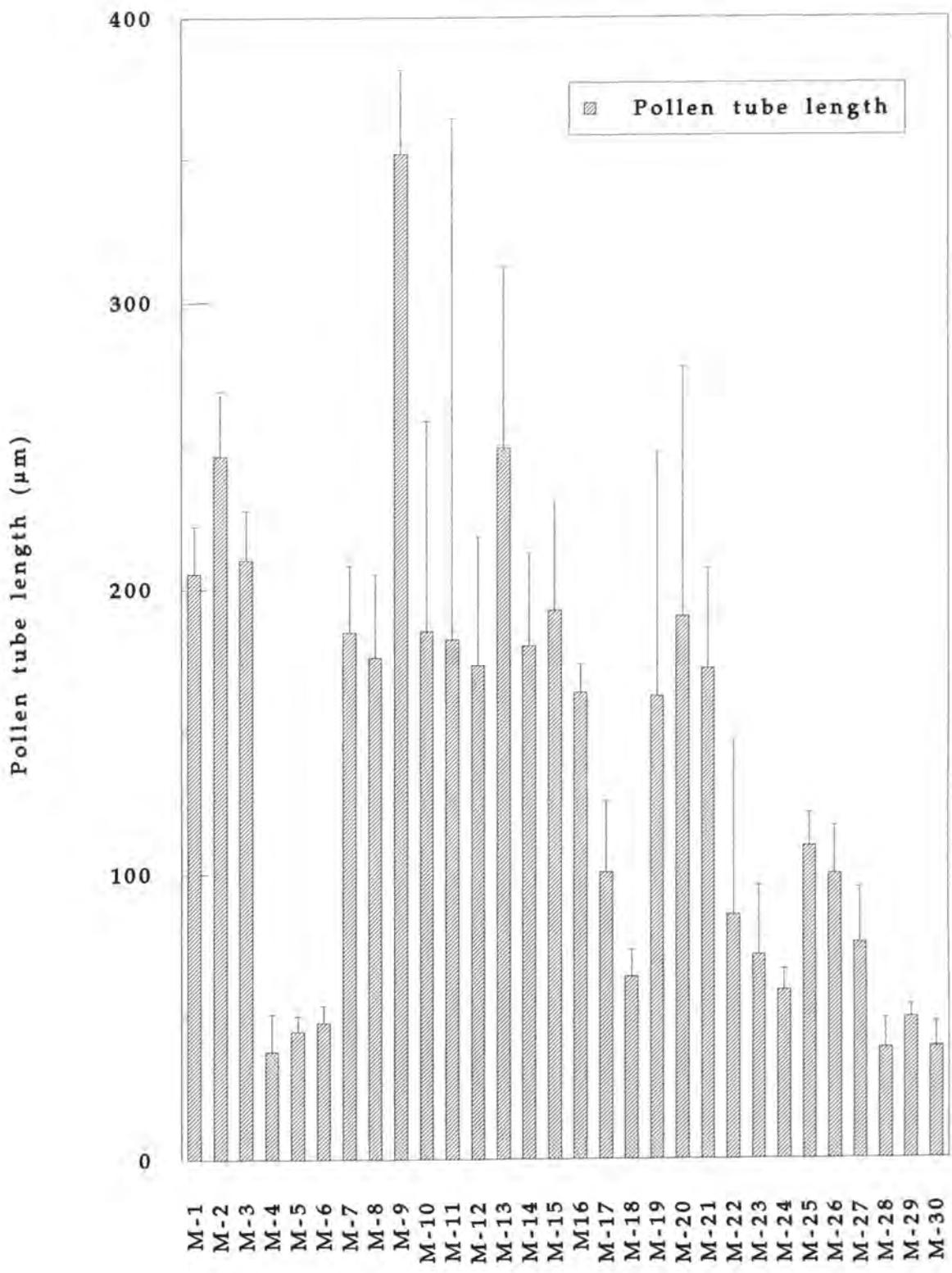


Fig.-3.12 Effect of different levels of boric acid and sucrose with fixed levels of calcium nitrate on pollen germination of *Cicer arietinum*.



(Pollen germinating media)

Fig. 3.13. Effect of different concentrations of boric acid and sucrose with fixed level of calcium nitrate on pollen tube length of *Cicer arietinum*.

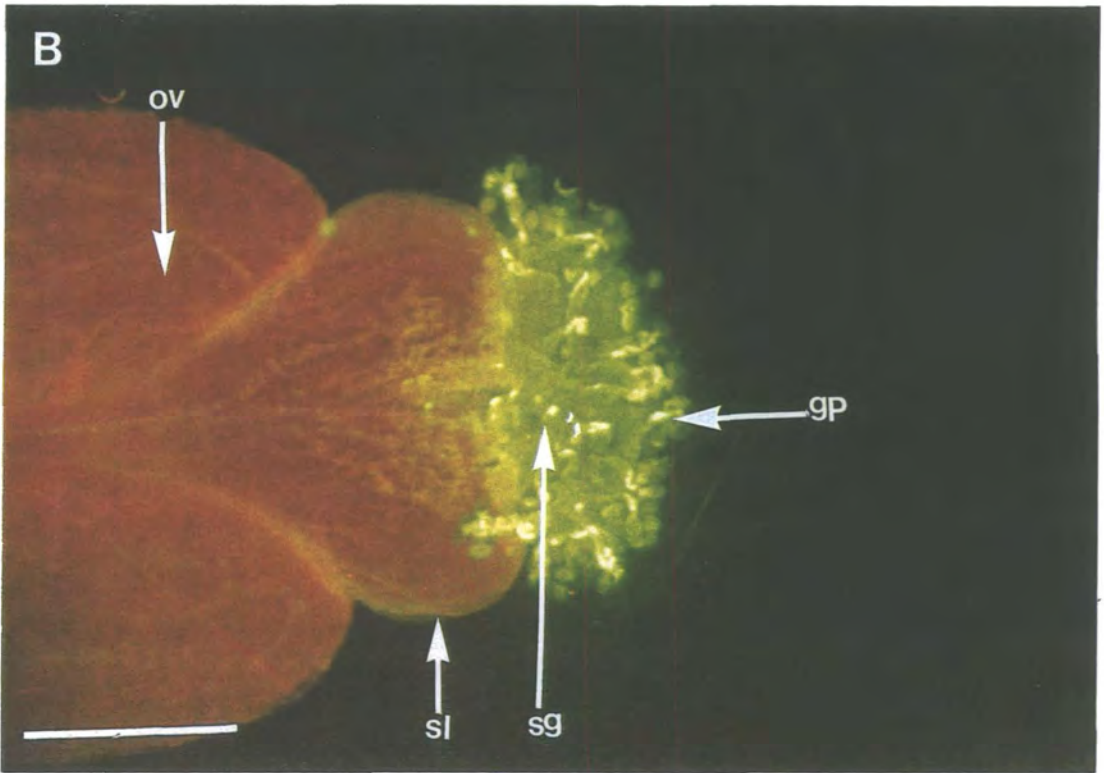
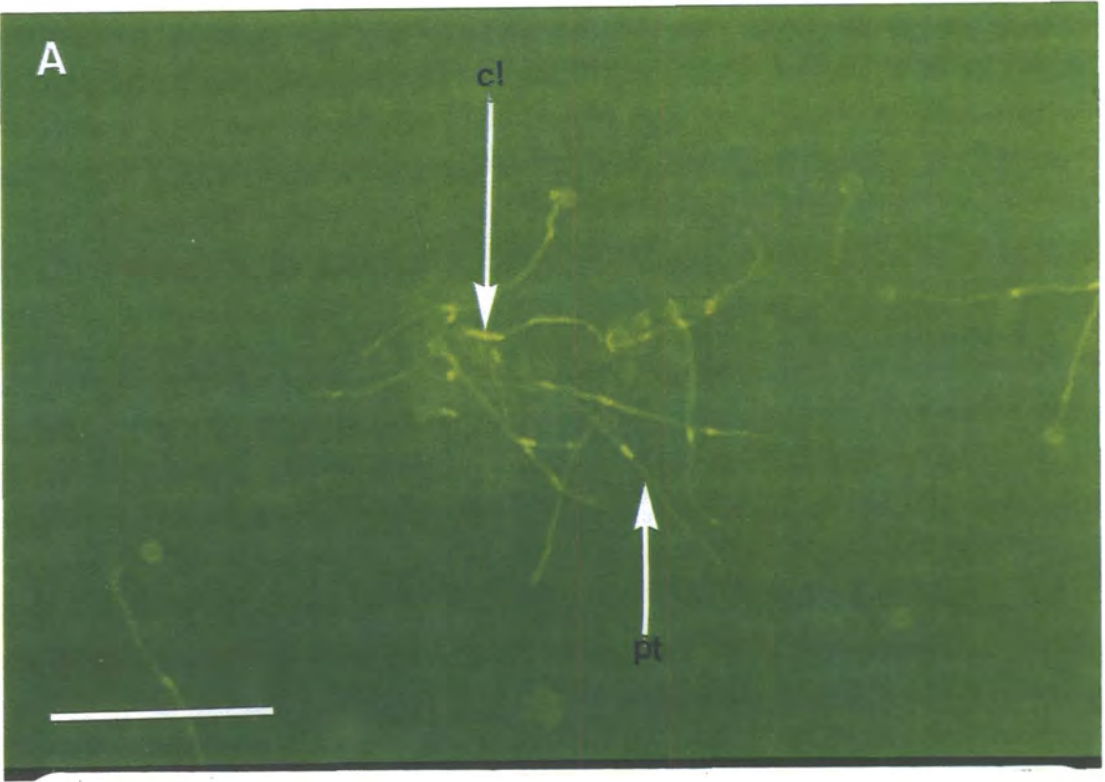


Plate 3.1 (A). *In vitro* pollen germination of *Arabidopsis thaliana* in the newly developed pollen germination medium (Table-3.1), notice the callose plugs in pollen tubes. Fluorescence micrograph. Aniline Blue Staining.

Plate 3.1 (B). *In vivo* pollen germination on the stigma of pistil of *A. thaliana*. Fluorescence micrograph. Aniline Blue Staining.

cl, callose plugs; pt, pollen tube; ov, ovary; gp, germinated pollen; sl, style; sg, stigma. (Scale bars = 200 μ m).

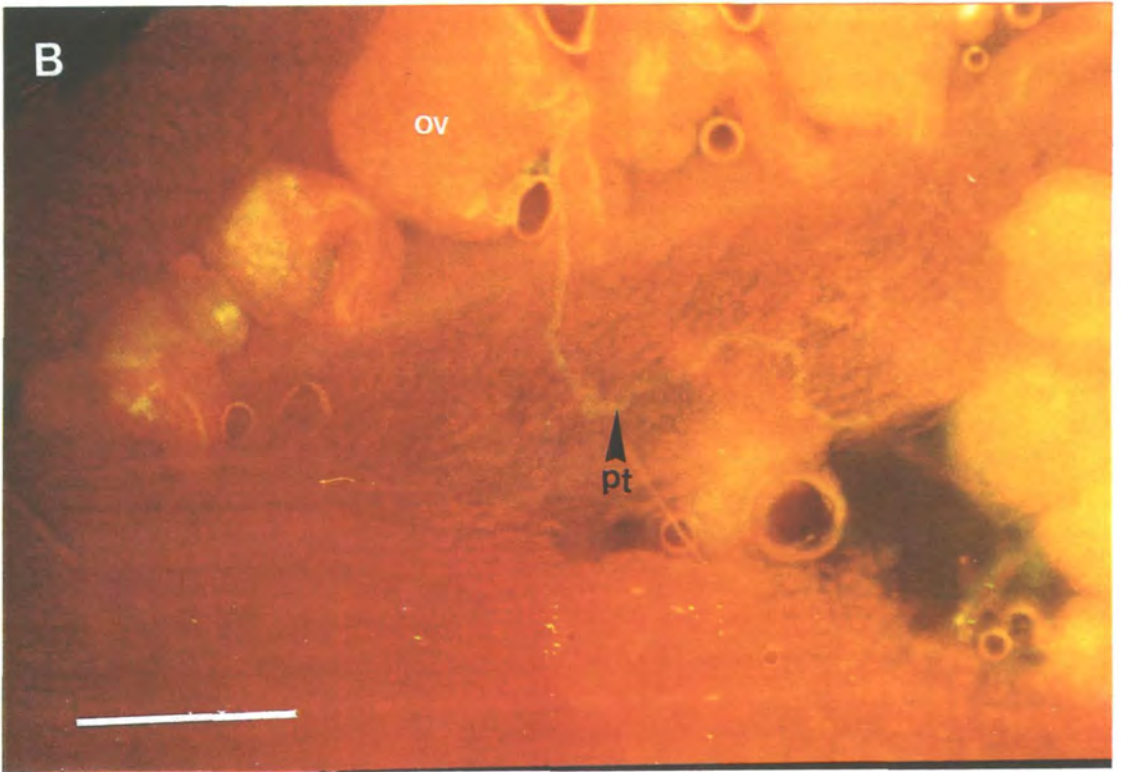
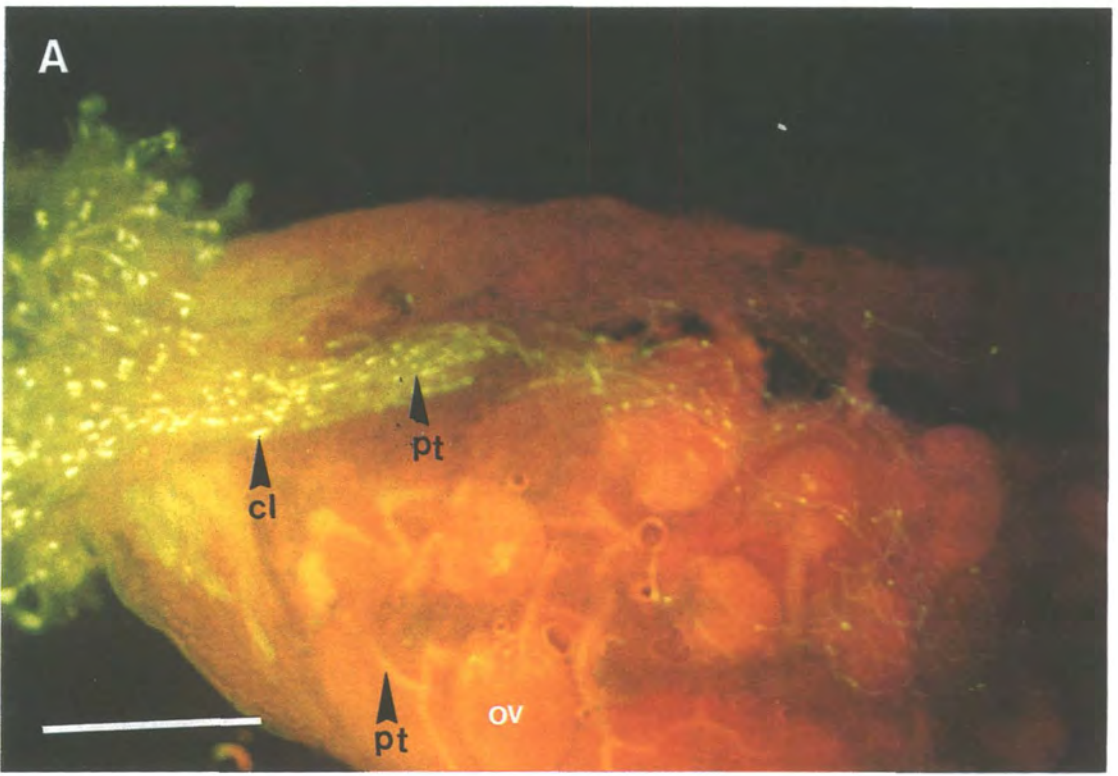


Plate 3.2 (A). *In vivo* growth of pollen in the style and the ovary of *Arabidopsis thaliana*. Fluorescence micrograph. Aniline Blue Staining.

Plate 3.2 (B). *In vivo* penetration of pollen tube into ovule. Fluorescence micrograph. Aniline Blue Staining.

cl, callose plugs; pt, pollen tubes; ov, ovule. (Scale bars = 100 μm).

Chapter IV

Effect of temperature on pollen germination and pollen tube growth in *Arabidopsis thaliana*

Three genotypes and eight temperature levels were taken in account to record the impacts of temperature on the pollen germination and growth of *Arabidopsis* pollen.

Statistically highly significant differences were calculated among temperature x genotypes ($P \leq 0.005$) regarding the pollen germination and growth.

4.0.1 Effects on pollen germination and pollen tube length

The pollen germination of all the genotypes was affected at high and low temperature regimes. The range of decline in pollen germination (Table-4.1, Fig. 4.1) varied from 94.21% to 91.18%, 93.07% to 93.43% and 89.27% to 91.20% under 0°C and 35°C in comparison to the control temperature (20°C) respectively, for genotypes NW-45, NW-46 and wildtype.

Like pollen germination, the pollen tube length was also affected by the low and high temperatures in all genotypes (Table-4.2, Fig. 4.2). The reductions in the pollen tube length of genotypes varied from 87.04% to 92.12%, 81.00% to 91.73% and 88.67% to 91.84% under incubated temperatures of 0°C and 35°C relatively to the control temperature (20°C) respectively, for genotypes NW-45, NW-46 and wild type.

4.1 *In vitro* rate of pollen germination and growth in *Arabidopsis*

In order to study the rate of pollen germination and tube growth under different temperature regimes, five time intervals (3, 5, 10, 15, 24 h) and eight temperatures (0, 5, 10, 15, 20, 25, 30, 35°C) were selected. Statistically highly significant differences were obtained for temperature treatments and time intervals ($p \leq 0.005$).

The pollen of *Arabidopsis* seemed to have a germination lag period of approximately one hour (observed during experimentation), and started germination thereafter. At 0°C the germination started after 10 h, at 5°C, after 5 h, while at all other temperatures it started after 1h.

The results (Table-4.3, 4.4, Fig. 4.3) indicate that under different incubation periods different calculated rates of pollen germination were obtained. The temperature 20°C produced higher per hour pollen germination percentage than all other temperatures under every time interval. It produced PG of 12.60%/h (3 h) to 3.55%/h (24 h). The PG after best temperature (20°C) was followed by 25°C, 15°C and 30°C which respectively produced PG of 8.80%/h- 2.52%/h, 7.52%/h-2.54/h and 3.99%/h-3.01%/h.

The calculated rate of pollen germination declined for increasing time intervals.

The results (Table-4.5, 4.6, Fig. 4.4) indicate that out of eight selected temperatures the highest per hour pollen tube growth rates were recorded under three incubated temperature regimes i.e. 20°C, 25°C and 30°C. Under 20°C incubation, the calculated per hour pollen tube growth rate was recorded for 5 h (60.86 $\mu\text{m}/\text{h}$) incubation period. While the pollen tube rates of 66.21 $\mu\text{m}/\text{h}$ and 48.83 $\mu\text{m}/\text{h}$ were recorded respectively for 25°C and 30°C temperature regimes under a 3 h incubation period. The rate of pollen tube growth as indicated in table 4.5 for

the remaining temperature regimes were far lower than high ranking temperature (20°C).

4.2 *In vivo* and *in vitro* PTL, comparison under different temperature levels

Comparing *in vivo* and *in vitro* pollen growth under different temperature regimes, non-significant ($P \leq 0.10$) differences among temperatures for both conditions were recorded. At every temperature level (Table-4.7, Fig. 4.5) pollen tube growth *in vivo* was much better than *in vitro*. The percent increase of *in vivo* over *in vitro* ranged from 67.97% (25°C) to 93.12% (10°C).

4.3 Effect of temperature on pollen germination and growth in *Cicer arietinum*

The data depicted (Table-4.8) state that highly significant differences for pollen germination ($f = 49.08$, $df = 5$, $P \leq 0.001$) and pollen tube length ($f = 28.13$, $df = 5$, $P \leq 0.001$) were noted when pollen collected from *Cicer arietinum* was incubated at different temperature regimes.

The germination slightly increased with the rising temperature from 10°C to 15°C. It attained its peak at 20°C (62.35%) and declined thereafter (Fig. 4.6). The highest tested temperature of 35°C was more harmful in inhibiting germination bringing down by 10.20%.

The pollen tube length also increased slowly (Fig. 4.7) from 10°C to 15°C but increased to reach a maximum at 20°C (358.32 μm). It started to decrease thereafter and declined to 83.88 μm at highest incubated temperature (35°C).

Table-4.1: Pollen germination in different genotypes of *A. thaliana* incubated under different temperature regimes for 24 hours.

Genoty	0°C	5°C	10°C	15°C	20°C (control)	25°C	30°C	35°C
	**	**	**	**	**	**	**	**
NW-45	4.75±2.75 (-94.21)	54.19±8.89 (-33.99)	66.29±6.82 (-19.26)	72.14±8.84 (-12.13)	82.10±4.09 -	58.14±4.04 (-29.18)	56.90±3.68 (-30.69)	4.24±0.25 (-91.18)
NW-46	4.68±1.19 (-93.07)	49.60±3.32 (-26.56)	50.88±5.94 (-24.67)	56.1±5.03 (-16.94)	67.54±6.71 -	57.25±2.57 (-15.23)	57.63±4.62 (-14.67)	14.44±0.51 (-93.43)
Wild type	8.53±0.98 (-89.27)	45.59±4.45 (-42.68)	60.82±6.15 (-23.35)	61.20±3.12 (-23.05)	79.54±11.00 -	59.63±2.50 (-12.45)	58.40±1.67 (-26.57)	6.39±0.75 (-91.20)

**= Significant $P \leq 0.005$

Note= In parenthesis % decrease (-) against control temperature (20°C) is given

Table-4.2: Pollen tube growth (μm) in different genotypes of *A. thaliana* incubated under different temperature regimes for 24 hours.

Genoty.	0°C	5°C	10°C	15°C	20°C (control)	25°C	30°C	35°C
	*	*	*	*	*	*	*	*
NW-45	83.97±10.90 (-87.04)	218.56±15.19 (-66.26)	450.74±39.85 (-30.41)	490.82±75.23 (-24.23)	647.76±62.30 -	702.32±45.91 (+8.42)	237.35±15.23 (-63.35)	51.04±5.71 (-92.12)
NW-46	98.83±6.23 (-81.00)	122.79±5.25 (-76.42)	341.86±35.25 (-34.35)	356.12±40.45 (-31.61)	520.71±50.00 -	613.86±45.60 (+17.88)	165.07±12.15 (-68.29)	43.04±8.92 (-91.73)
Wild	65.59±5.71 (-88.67)	179.81±6.25 (-68.93)	345.55±25.71 (-40.29)	457.70±48.00 (-20.92)	578.80±42.00 -	686.59±40.00 (+18.62)	301.78±34.65 (-47.86)	47.22±12.11 (-91.84)

*= Significant $P \leq 0.05$

Note= Figures in parenthesis indicate percent decrease (-) or increase (+) of experimental treatment over control

Table-4.3: *In vitro* pollen germination in *A. thaliana* (wild type) incubated under different temperature regimes at different time intervals.

Temperature	3 h	5 h	10 h	15 h	24 h
0°C	*** ng	*** ng	*** ng	*** 6.0	*** 7.89
5°C	ng	ng	23.48	36.92	43.05
10°C	3.0	11.04	35.0	57.47	62.11
15°C	22.56	28.43	39.03	54.35	61.06
20°C	37.80	60.56	75.29	83.69	85.30
25°C	26.41	43.22	50.27	56.73	60.50
30°C	11.98	40.55	68.00	69.65	72.27
35°C	1.8	4.6	4.71	5.08	7.71

***= Significant $P \leq 0.005$

ng denotes no germination



Table-4.4: Calculated rates of pollen germination percentage per hour in *A. thaliana* (wild type) incubated under different temperature regimes at different time intervals.

Temperature	3 h	5 h	10 h	15	24 h
	***	***	***	***	***
0°C	ng	ng	ng	0.40	0.33
5°C	ng	ng	2.35	2.46	1.79
10°C	1.00	2.21	3.50	3.83	2.59
15°C	7.52	5.69	3.90	3.62	2.54
20°C	12.60	12.11	7.53	5.58	3.55
25°C	8.80	8.64	5.02	3.78	2.52
30°C	3.99	8.11	6.80	4.64	3.01
35°C	0.60	0.92	0.47	0.34	0.32

***= Significant $P \leq 0.005$

ng denotes no germination

Table-4.5: *In vitro* rate of pollen tube growth (μm) in *A. thaliana* (wild type) incubated under different temperature regimes at different time intervals.

Temperature	3 h	5 h	10 h	15 h	24
	***	***	***	***	***
0°C	ng	ng	ng	50.00	58.47
5°C	ng	ng	79.24	101.36	173.91
10°C	45.00	138.78	142.23	270.52	329.74
15°C	80.60	157.30	308.41	414.70	430.50
20°C	122.46	304.30	405.75	528.87	654.27
25°C	198.63	238.17	339.69	576.35	674.33
30°C	146.50	168.52	189.57	287.33	295.32
35°C	25.45	45.0	45.32	46.21	47.61

***= Significant $P \leq 0.005$

ng= No germination

Table-4.6: Calculated rate of pollen tube growth ($\mu\text{m}/\text{h}$) in *A. thaliana* (wild type) incubated under different temperature regimes at different time intervals.

Temperature	3 h	5 h	10 h	15 h	24 h
0°C	-	-	-	3.33	2.43
5°C	-	-	7.92	6.76	7.24
10°C	15.0	27.76	14.22	18.03	13.74
15°C	26.87	31.46	30.84	27.65	17.93
20°C	40.82	60.86	40.57	35.26	27.26
25°C	66.21	47.63	33.96	38.42	28.09
30°C	48.83	33.70	18.95	19.15	12.30
35°C	8.48	5.09	4.53	3.08	1.98

Table-4.7: *In vivo* and *in vitro* comparison of pollen tube growth (μm) under different temperature regimes in *A. thaliana* (wild type).

Temperature	<i>In vivo</i>	<i>In vitro</i>	% increase over <i>in vitro</i>
	ns	ns	
10°C	654.09±100.50	45.0±10.11	93.12
15°C	601.80±150.45	80.60±19.90	86.60
20°C	656.90±122.15	122.46±67.30	81.35
25°C	620.08±95.23	198.63±39.20	67.97
30°C	671.80 ±135.20	146.50 ±20.70	78.19

ns= Non significant

Table-4.8: Pollen germination and growth in *Cicer-arietinum* incubated under different temperature regimes for 24 hour.

Temperature	Pollen Germination (%)	Pollen tube length (μm)
	***	***
10°C	46.91±12.70	252.01±50.63
15°C	48.56±16.15	261.34±65.95
20°C	62.35±19.64	358.32±49.22
25°C	30.87±5.87	255.58 ±33.67
30°C	20.71±3.92	146.75±40.19
35°C	10.20±2.21	83.88±30.23
F (df)	49.08 (5)	28.13 (5)
P ≤	0.001	0.001

***= significant $P \leq 0.005$

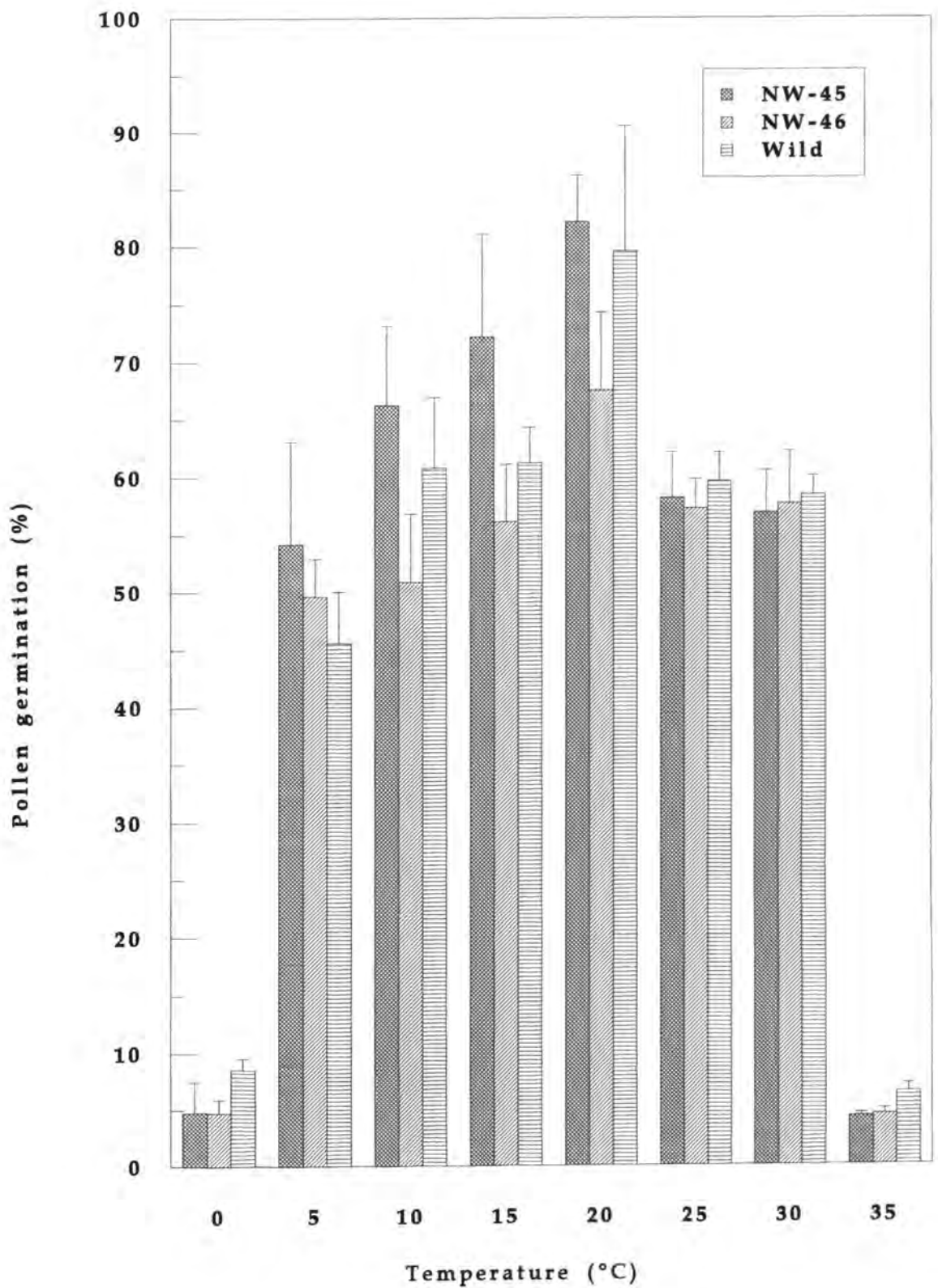


Fig. 4.1. Pollen germination of different genotypes of *A. thaliana* under different temperature regimes.

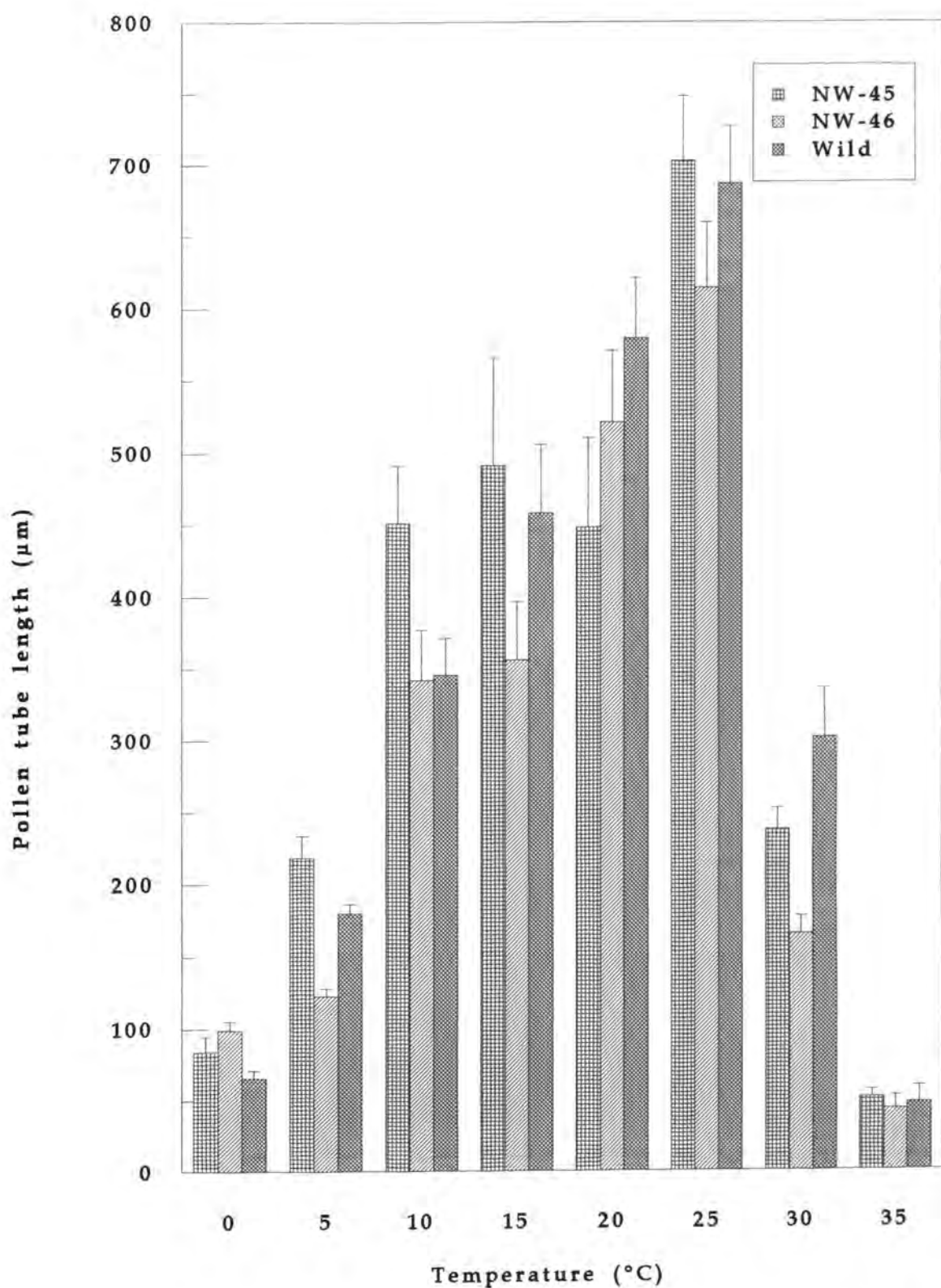


Fig. 4.2. Pollen tube length of different genotypes of *A. thaliana* under different temperature regimes (incubated for 24 hours).

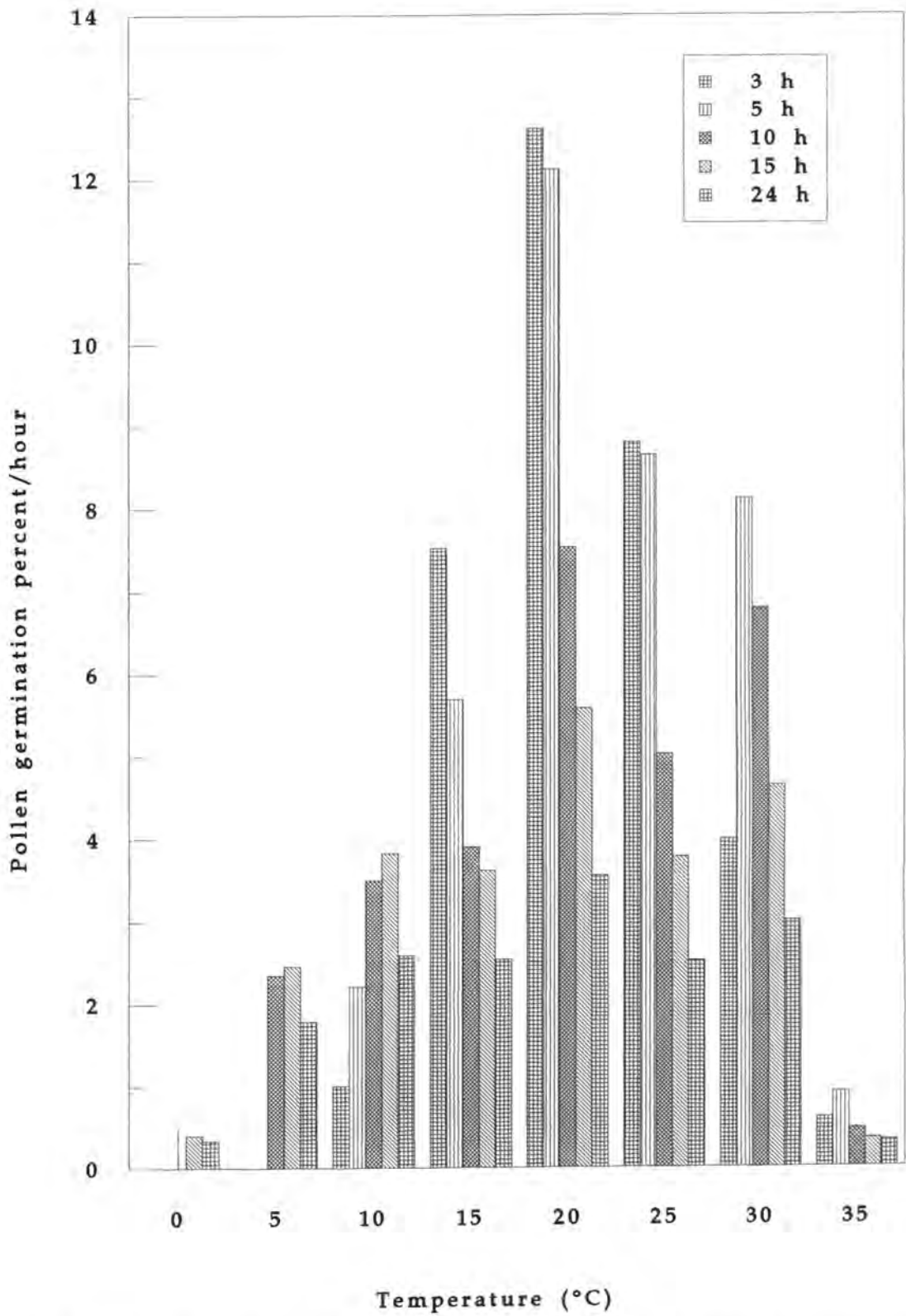


Fig. 4.3. Calculated rates of pollen germination percentage per hour in *A. thaliana* (wild type) incubated under different temperature regimes at different time intervals.

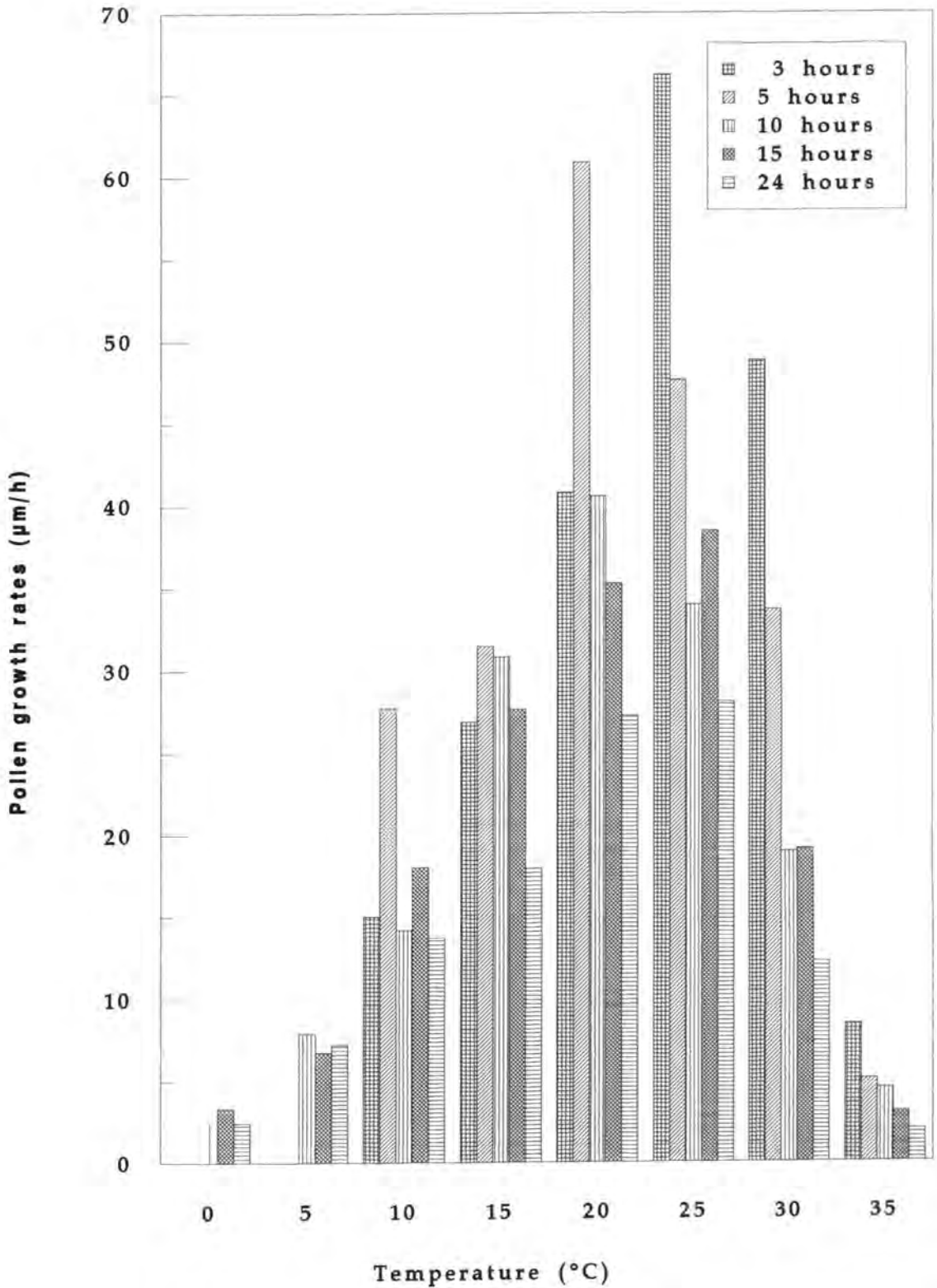


Fig. 4.4. Pollen growth rates ($\mu\text{m/h}$) in *A. thaliana* (wild type), incubated under different temperature regimes at different time intervals.

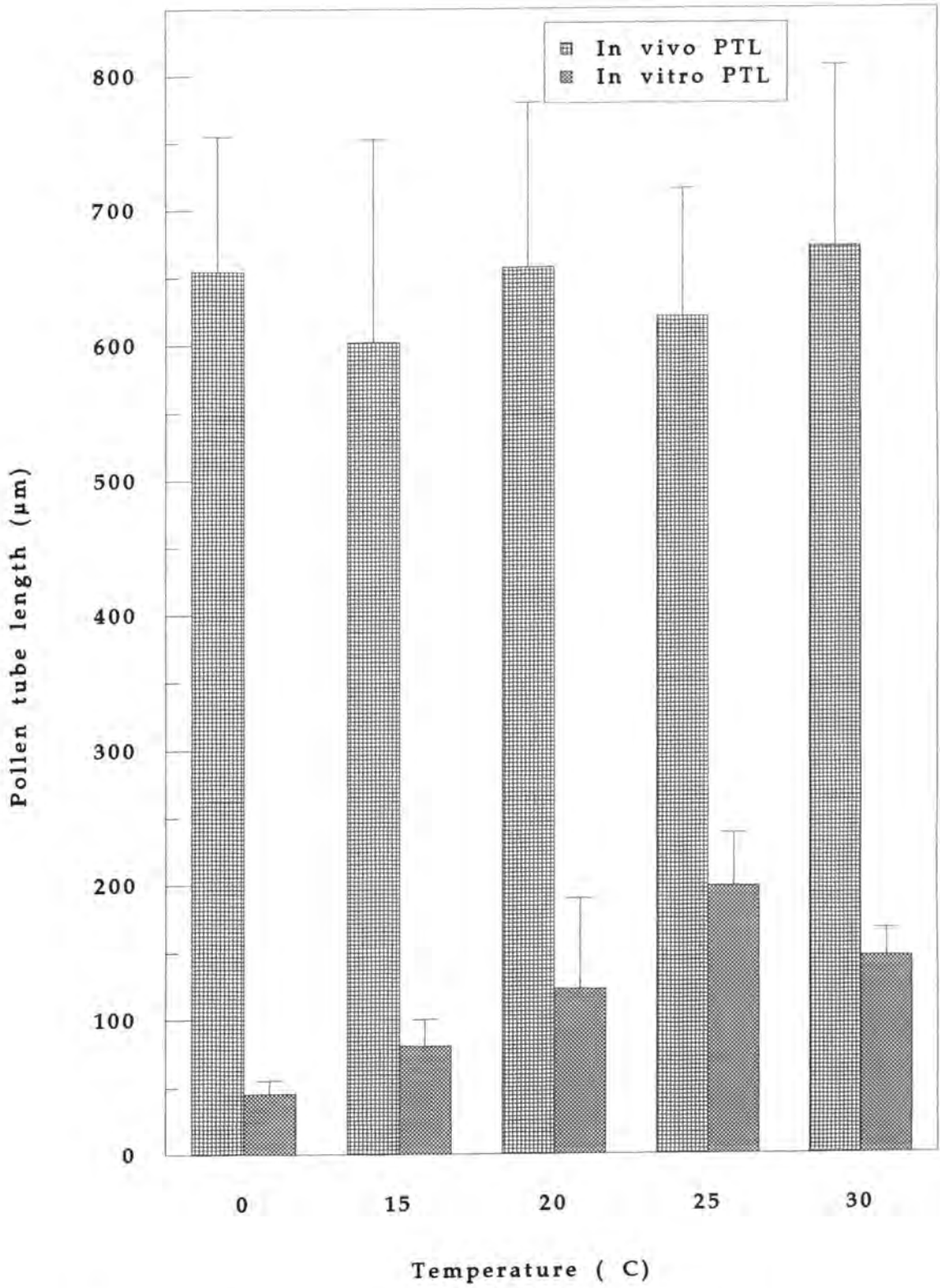


Fig. 4.5. In vivo and in vitro comparison of pollen tube growth (μm) under different temperature regimes in *A. thaliana* (wild type) incubated for 3 hours.

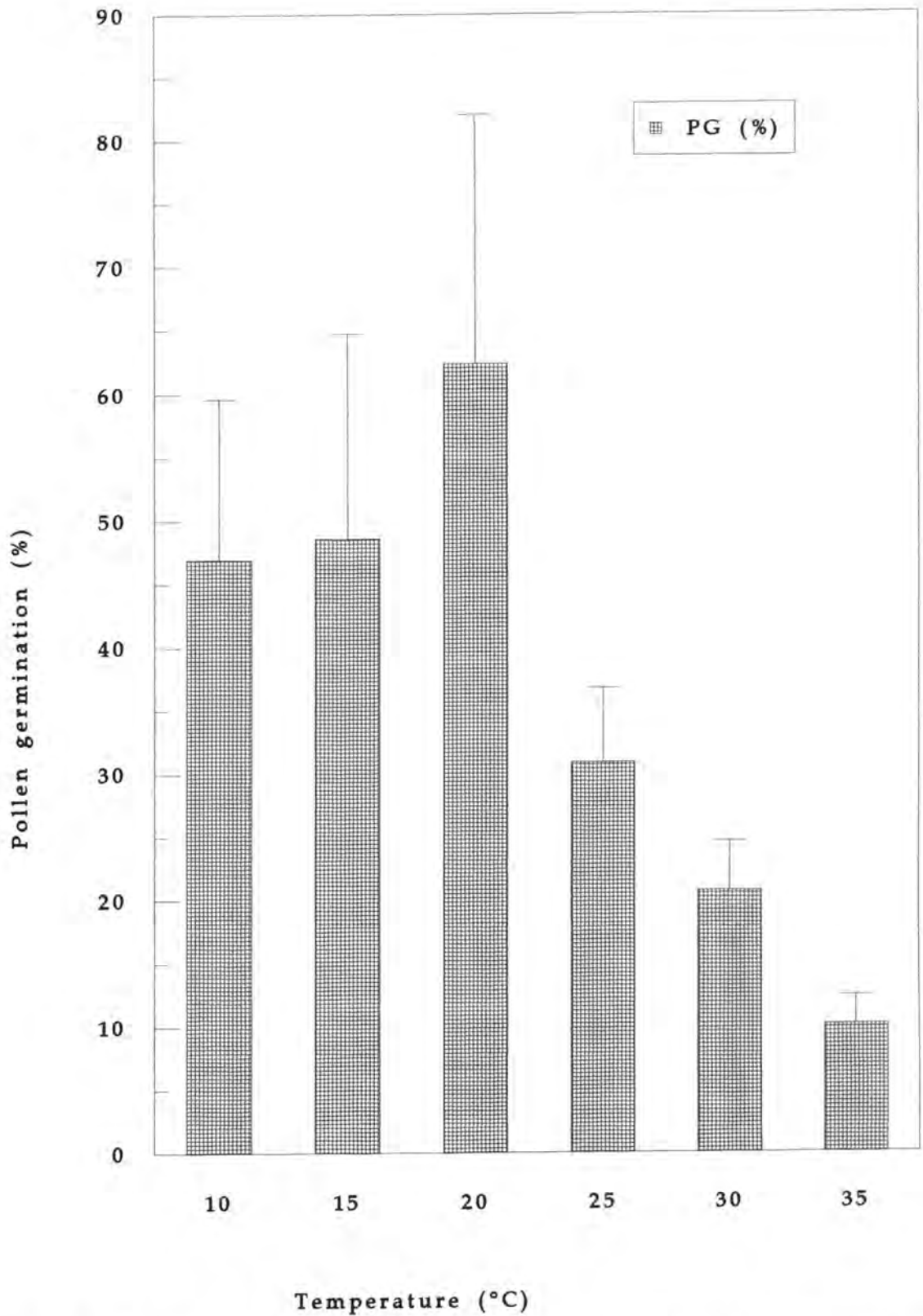


Fig. 4.6. Pollen germination (%) of *C. arietinum* pollen, incubated for 24 hours under different temperature regimes.

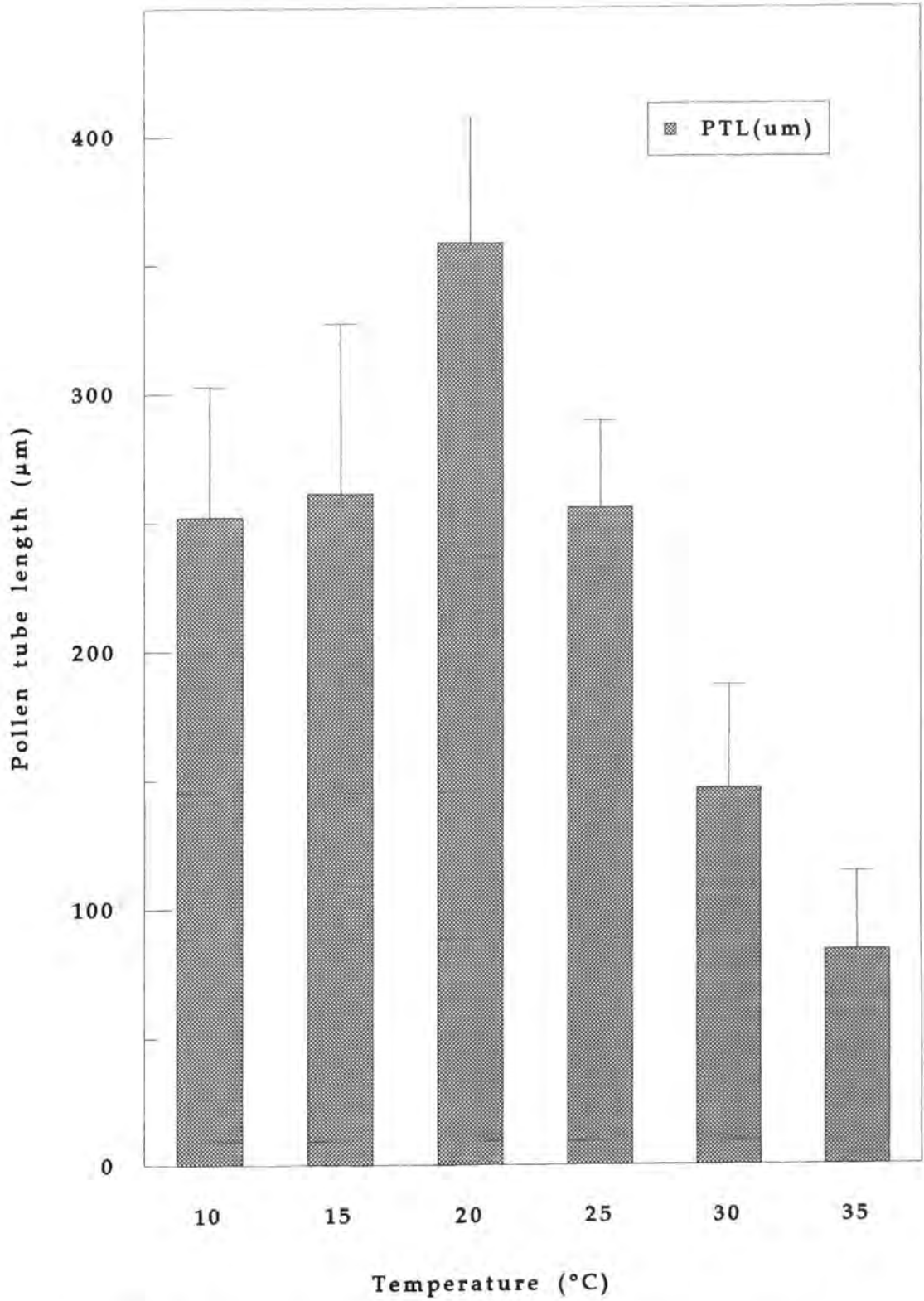


Fig. 4.7. Pollen tube length (μm) of *C. arietinum* pollen, incubated for 24 hours under different temperature regimes.

Chapter V

Impact of differential pollen tube growth rates on the sporophytic traits of *Arabidopsis thaliana*

As described in material and methods, a common male sterile (MS) female parent was chosen to cross with pollen donors having differential pollen growth rates. For pollen donors and hybrids the 'wild type' and 'MS x wild type' were used as controls. Highly significant differences, between various male parents, from cross pods for number of seeds/pod ($f = 3.30$, $df = 9$, $p \leq 0.01$) and filled seeds/pod ($f = 5.32$, $df = 9$, $p \leq 0.001$) were obtained. Comparing the pollen donors and hybrids with above controls, increases (Table-5.1, Fig. 5.1, 5.2), ranging from 2.37% (MS x NW-91) to 30.95 % (CAD1) and from 4.76 (MS x NW-91) to 30.49 % (CAD1) were recorded for number of seeds/pod and filled seeds respectively.

F1s

In F1s, PTGRs and eight sporophytic traits were investigated to determine how these were influenced by the differential pollen growth rates of pollen donors. The results obtained showed statistically variable trends. The character-wise results are given below.

5.1 Pollen growth rates (PTGRs)

Highly significant differences ($f = 66.13$, $df = 9$, $p \leq 0.001$) among pollen donors and crosses were recorded in respect to PTGRs. Comparing with controls (Table-5.2, 5.3, Fig. 5.3) the pollen donors (PDs) produced higher pollen growth

rates varying from 1.91% (NW-91) to 119.81 % (CAD1) compared with wild type. The crosses gave 1.75% (MS x NW-28) to 85.64 % (MS x CAD1) greater PTGRs compared with MS x Wild type (control). A decrease of 17.41 % was observed for in MS x NW-46 compared with the control. Comparing the hybrids with their pollen donors (Table-5.4), only one hybrid (MS x NW-91) surpassed its pollen donor for PTGRs. All other hybrids remained below the range of their pollen parents.

5.2 Rosette diameter (RD)

Non-significant results for rosette diameter were obtained ($f = 2.57$, $df = 9$.) Comparing F1 with controls (Table -5.2, 5.3, Fig. 5.4), it was observed that the RD non-significantly increased in crosses involving pollen donors NW-28 (1.60 %) and CAD1 (44.74 %) and decreased in NW-46 (20.68 %) and NW-28 (1.60 %). In the case of hybrid comparisons the data indicate that only one hybrid MS x NW-46 (13.46 %) exhibited a decline, while all other hybrids produced increases ranging from 1.08% (MS x NW-28) to 33.74 % (MS x CAD1) compared with the control MS x Wild.

Comparing the hybrids (Table-5.4) with their pollen donors, 11.07% (MS x NW-91) to 42.02 % (MS x NW-46) increase and 0.71% (MS x Wild type) to 8.25 % (MS x CAD1) decline was recorded in hybrids.

Plant height

Highly significant ($f = 8.52$, $df = 9$, $p \leq 0.01$) differences were found for plant height (PH) among donors and F1s. Most of the donors showed a decline in plant height (Table-5.2, 5.3, Fig. 5.8) compared with the control, while only one

genotype (CAD1) gave taller plant height relatively to the control. In the case of hybrids three gave increases ranging from 0.91% (MS x NW-46) to 56.52 % (MS x CAD1), while PH declined in one hybrid MS x NW-91 by 7.23 % compared with the control.

Comparing donor parents (Table-5.4) with hybrids, three hybrids gave greater plant height, ranging from 5.75% (MS x NW-91) to 44.27 % (MS x NW-91). The remaining two hybrids gave 3.74% (MS x Wild) to 13.68 % (MS x CAD1) lower plant heights than their pollen donors.

5.3 Branches/plant (B/P)

Significant differences ($f = 3.99$, $df = 9$, $p \leq 0.05$) were recorded among hybrids and pollen donors in respect of branches/plant. Mixed trends of increase and decrease were noted (Table-5.2, 5.3, Fig. 5.5) in comparison with controls. The increases varied from 0.67 (CAD1) to 51.35 % (NW-28), while decline in B/P varied from 8.95% (MS x NW-91) to 51.13 % (NW-46).

Comparing crosses (Table-5.4) with pollen donors, 6.04% (MS x CAD1) to 66.67 % (MS x NW-46) increases and 9.46 (MS x Wild type) to 34.82 % (MS x NW-28) declines were recorded.

5.4 Pods/plant (P/P)

Significant ($f = 4.66$, $df = 9$, $p \leq 0.05$) differences (Table-5.2, 5.3) for the pods/plant were noted. The increase in pods (Table -5.3) varied from 15.79% (MS x NW-28) to 39.29 % (MS x CAD1), while decrease ranged from 30.97% (MS x NW-46) to 60.85 % (NW-91).

Comparing hybrids with pollen donors (Table-5.4, Fig. 5.5) increases in pods/plant ranging from 23.42% (MS x NW-91) to 78.65% (MS x NW-28) and declines from 0.76% (MS x CAD1) to 8.61% (MS x Wild) were recorded.

5.5 Earliness

The parameters of earliness taken in account varied nonsignificantly for pollen donors and F1s. The details of results are shown below.

Raceme initiation (RI)

The data (Table-5.5, Fig. 5.6), indicates that two pollen donors, NW-46 (4.40 %) and NW-28 (0.27 %), had non-significantly ($f = 0.85$, $df = 9$) earlier raceme initiation, while the remaining two pollen donors NW-91 (8.60 %) and CAD1 (0.80 %) had later racemes. In the case of the F1s, the crosses MS x NW-46 (2.94 %) and MS x NW-28 (3.75 %) were earlier than the control, while the remaining two crosses, MS x NW-91 (2.94 %) and MS x CAD1 (1.61) initiated late racemes.

Comparing the performance of hybrids with pollen donors (Table-5.6) it is evident that two hybrids, MS x NW-28 (3.23%) and MS x NW-91 (4.95 %), yielded early racemes; on the other hand, the remaining three hybrids initiated racemes from 0.27% (MS x Wild) to 1.40 % (MS x NW-46) later.

5.5.1 First flower

Non-significant differences were detected ($f = 2.13$, $df = 9$) for early flowering, ranging from 0.49% (MS x NW-46) to 3.25 % (NW-26) among pollen donors (Table-5.5, Fig. 5.6) and hybrids. Late flower emergence, ranging from 0.75% to 9.25 % was recorded respectively in MS x CAD1 and NW-91.

5.5.2 Days from raceme initiation to flowering

Most of the donors and hybrids took non-significantly more days ($f = 1.14$, $df = 9$) from raceme initiation to flowering (Table-5.5, Fig. 5.6). Only the pollen donor NW-46 (6.67 %) was slightly earlier than the control wild type.

Comparing the calculated values of hybrids and pollen donors (Table-5.6), only one hybrid, MS x NW-46 (35.71 %) took longer than its pollen donor to flower.

5.5.3 Plant dry weight (PDW)

Highly significant ($f = 6.73$, $df = 9$, $p \leq 0.01$) differences were recorded for plant dry weight among pollen donors and F1s. The PDW was stimulated among all F1 hybrids (Table-5.2-5.3, Fig. 5.7) from 32.90% (MS x NW-91) to 134.19% (MS x CAD1) in comparison to the control.

5.6 Correlations

Comparing the correlations of PTGRs of pollen donors with sporophytic traits, a very loose correlation (+0.085) was recorded for raceme initiation and negative correlations were recorded for other earliness characters i.e. days to flower (-0.17) and days from raceme to flowering (-0.03). For rest of the sporophytic traits viz. rosette diameter (+0.92), pods/plant (+0.82), branches/plant (+0.79), plant height (+0.98) and plant dry weight (+0.76) strong correlation were recorded.

Table-5.1: Total number of seeds set/pod and filled seed (%) in *A. thaliana* after crossing male-sterile with different pollen donors. The hybrids compared with control, MS x wild type and pollen donors with control, wild type, SD in parenthesis.

Combinations	Seeds set/pod	% increase	Filled seed (%)	% increase
MS x NW-46	35.20 (4.44)	4.41	81.22 (7.55)	13.48
MS x NW-28	37.0 (2.42)	9.47	83.74 (5.54)	17.00
MS x NW-91	34.6 (8.56)	2.37	74.98 (7.00)	4.76
MS x CAD1	37.4 (2.70)	10.65	89.96 (8.11)	25.69
MS x Wild type (control)	33.80 (4.95)	-	71.57 (9.16)	-
NW-46	29.80 (5.0)	18.25	81.37 (6.19)	14.78
NW-28	31.40 (5.55)	24.60	87.89 (6.00)	18.12
NW-91	27.00 (4.18)	7.14	77.87 (10.13)	9.84
CAD1	33.00 (2.12)	30.95	92.51 (10.97)	30.49
Wild type (control)	25.20 (5.93)	-	70.89 9.37	-
F(df)	3.30 (9)	-	5.32 (9)	-
P _≤	0.01	-	0.001	-

Table-5.2: Mean values of **PTGRs** (5 hour incubation) and **sporophytic** traits in F1s after crossing male-sterile with different pollen donors, in *A. thaliana*. SD in parenthesis.

Combinations	PTGRs (μm)	Rosette diameter (cm)	plant height (cm)	Branches per plant	Pods per plant	Plant dry wt. (g)
MS x NW-46	221.13 (27.07)	6.32 (1.12)	22.05 (6.72)	6.00 (4.81)	56.40 (28.28)	0.4443 (0.21)
NW-46	379.52 (10.70)	4.45 (0.15)	17.79 (4.06)	3.60 (0.56)	57.73 (45.14)	0.1653 (0.10)
MS x NW-28	272.45 (71.13)	5.63 (0.51)	21.90 (3.82)	7.30 (0.42)	94.60 (19.79)	0.2831 (0.15)
NW-28	379.00 (24.56)	5.70 (1.5)	20.71 (2.99)	11.20 (3.96)	52.95 (36.84)	0.2848 (0.11)
MS x NW-91	292.31 (53.80)	5.82 (2.14)	20.27 (7.17)	6.1 (4.10)	43.20 (22.06)	0.2589 (0.11)
NW-91	285.49 (21.21)	5.24 (0.88)	14.05 (1.70)	5.0 (0.00)	35.0 (1.27)	0.0706 (0.02)
MS x wild type (control)	267.76 (47.99)	5.57 (0.14)	21.85 (6.15)	6.7 (0.99)	81.70 (26.73)	0.1948 (0.07)
Wild type (control)	280.14 (23.41)	5.61 (0.59)	22.70 (2.83)	7.40 (1.69)	89.40 (51.76)	0.3110 (0.19)
MS x CAD1	497.08 (33.13)	7.45 (2.04)	34.20 (4.67)	7.9 (0.14)	113.80 (21.50)	0.4562 (0.01)
CAD1	615.78 (68.04)	8.12 (0.36)	39.59 (10.82)	7.45 (0.07)	114.67 (44.21)	0.668 (0.12)
F (df)	66.13 (9)	2.57 (9)	8.52 (9)	3.99 (9)	4.66 (9)	6.73 (9)
P \leq	0.001	ns	0.01	0.05	0.05	0.01

Table-5.3:- Percent increase (+) or decrease (-) of pollen donors and crosses over controls for pollen growth rates and sporophytic traits in *Arabidopsis thaliana*.

Combinations	PTGRs (μm)	Rosette diametre (cm)	Plant height (cm)	Branches per plant	Pods per plant	Plant dry wt. (g)
MS x NW-46	-17.41	-13.46	+0.91	-10.45	-30.97	+128.08
NW-46	+35.47	-20.68	-21.62	-51.13	-35.42	-46.85
MS x NW-28	+ 1.75	+1.08	+0.23	+8.95	+15.79	+45.32
NW-28	+41.71	+1.60	-8.77	+51.35	-40.77	-8.42
MS x NW91	+9.17	+4.49	-7.23	-8.95	-47.12	+32.90
NW- 91	+1.91	-6.59	-38.10	-32.43	-60.85	-77.29
MS x wild type (control)	-	-	-	-	-	-
Wild type (control)	-	-	-	-	-	-
MS x CAD1	+85.64	+33.74	+56.52	+17.91	+39.29	+134.19
CAD1	+119.81	+44.74	+74.40	+0.67	+28.26	+114.82

Table-5.4: Percent increase (+) or decrease (-) of hybrids against their pollen donors in *Arabidopsis thaliana* in respect of pollen growth rates and sporophytic traits.

Combinations	PTGRs (μm)	Rosette diametre (cm)	Plant height (cm)	Branches per plant	Pods per plant
MS x NW-46	-41.73	+42.02	+23.94	+66.67	-2.30
NW-46	-	-	-	-	-
MS x NW-28	-31.37	-1.23	+5.75	-34.82	+78.65
NW-28	-	-	-	-	-
MS x NW-91	+2.39	+11.07	+44.27	+22.00	+23.42
NW-91	-	-	-	-	-
MS x wild type (control)	-4.42	-0.71	-3.74	-9.46	-8.61
Wild type (control)	-	-	-	-	-
MS x CAD1	-19.28	-8.25	-13.68	+6.04	-0.76

Table-5.5: Earliness performance of sporophytes, after crossing the male-sterile with different pollen donors in *Arabidopsis thaliana*. SD in parenthesis.

Combinations	Raceme initiation (DAP)	% inc. (+) or dec. (-)	First flower (DAP)	% inc.(+) or dec. (-)	Days from raceme to flowering	% inc. (+) or dec. (+)
MS x NW-46	36.20 (0.00)	-2.94 -	40.0 (0.85)	-0.49 -	3.80 (0.85)	+31.03 -
NW-46	35.70 (0.42)	-4.40 -	38.70 (0.42)	-3.25 -	2.80 (0.28)	-6.67 -
MS x NW-28	35.90 (0.99)	-3.75 -	39.32 (1.31)	-2.19 -	3.0 0.00	+15.38 -
NW-28	37.10 (0.99)	-0.27 -	40.50 (1.84)	-1.25 -	3.5 (0.99)	+16.67 -
MS x NW-91	38.40 (3.39)	+2.94 -	41.67 (2.72)	+3.66 -	2.90 (0.14)	+11.54 -
NW-91	40.40 (0.56)	+8.60 -	43.70 (0.42)	+9.25 -	3.30 (0.14)	+10.00 -
MS x wild type (control)	37.30 (1.84)	- -	40.20 (2.83)	- -	2.90 (0.99)	- -
Wild type (control)	37.20 (0.56)	- -	40.0 (0.56)	- -	3.0 (0.28)	- -
MS x CAD1	37.90 (3.25)	+1.61 -	40.50 2.12	+0.75 -	2.60 1.31	-10.34
CAD1	37.50 (0.71)	+0.80 -	41.80 (1.13)	+4.50 -	4.30 0.42	+43.33 -
F (df)	0.85 (9)	-	2.13 (9)	-	1.14 (9)	-
P ≤	ns	-	ns	-	ns	-

Table-5.6: Percent increase (+) or decrease (-) of hybrids against their pollen donors in *Arabidopsis thaliana* with respect to earliness performance.

Combinations	Raceme initiation (DAP)	First flower (DAP)	Days from raceme to flower
MS x NW-46	+1.40	+3.36	+35.71
NW-46	-	-	-
MS x NW-28	-3.23	-2.91	-14.28
NW-28	-	-	-
MS x NW-91	-4.95	-4.64	-12.12
NW-91	-	-	-
MS x wild type (control)	+0.27	+0.50	-3.33
Wild type (control)	-	-	-
MS x CAD1	+1.07	-3.11	-39.53
CAD1	-	-	-

DAP = Days after planting.

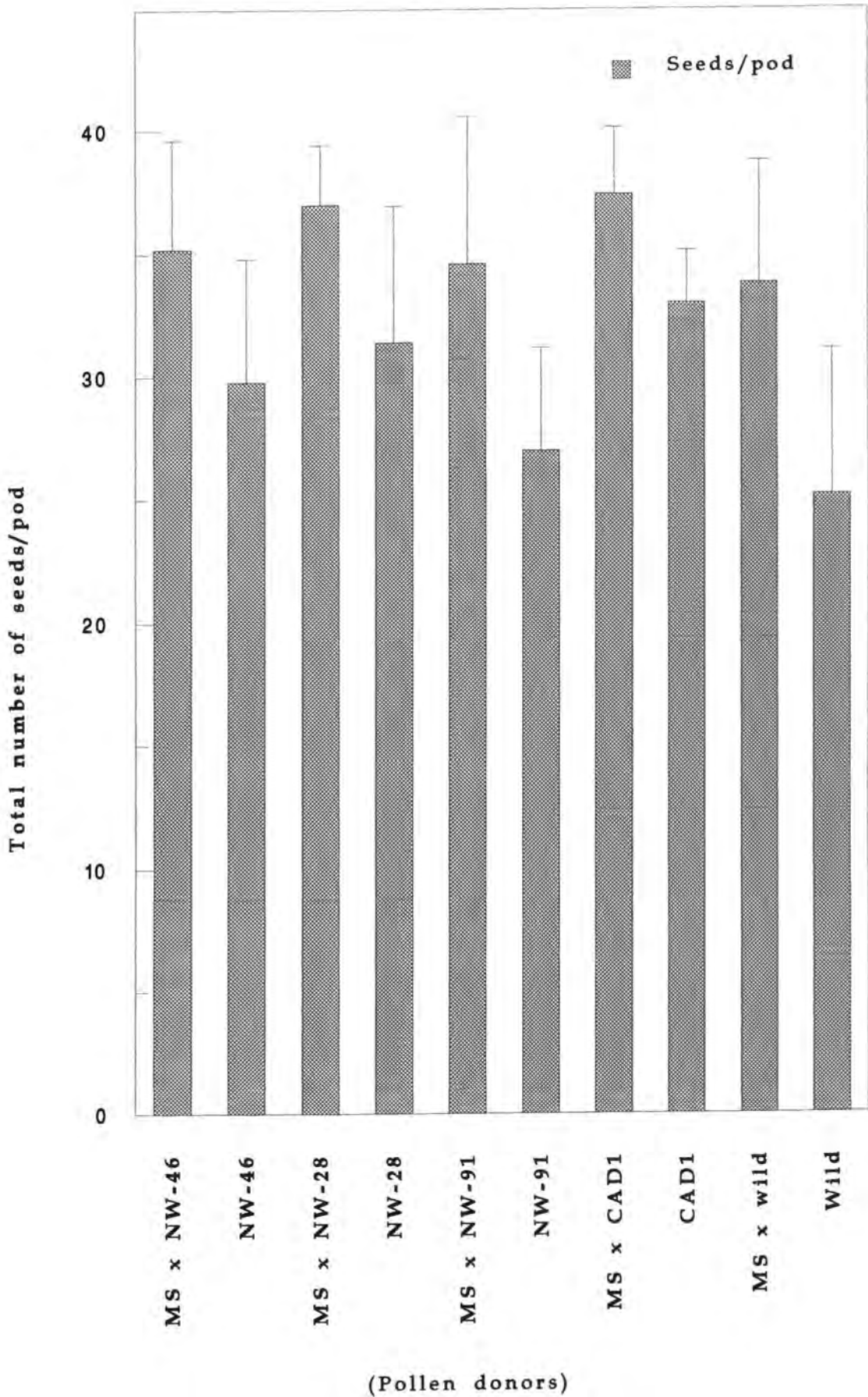


Fig. 5.1. Total number of seeds/pod in pollen donors and recipient-donor crosses in *Arabidopsis thaliana*.

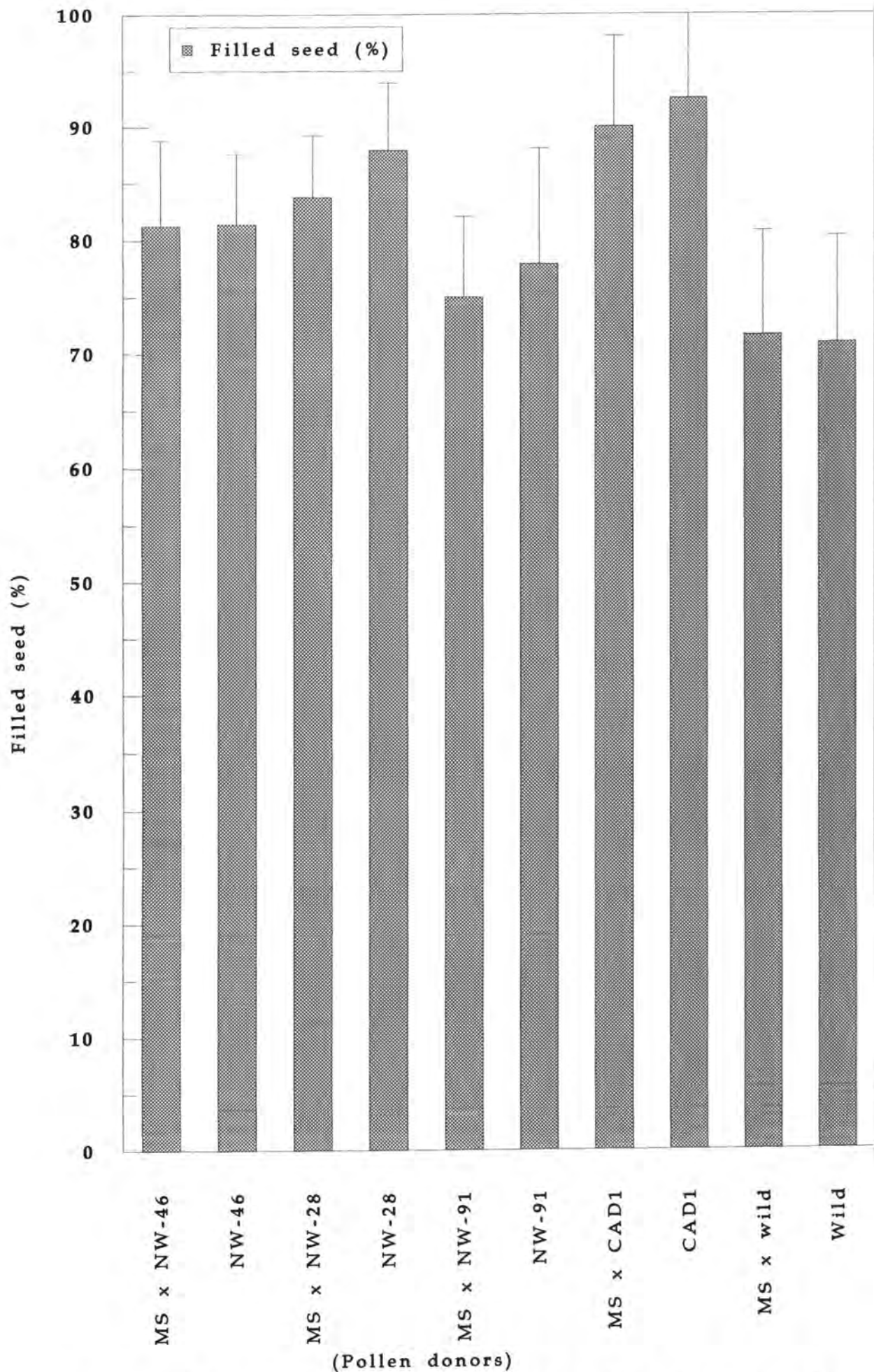


Fig. 5.2. Filled seed percentage in pollen donors and recipient-donor crosses in *Arabidopsis thaliana*.

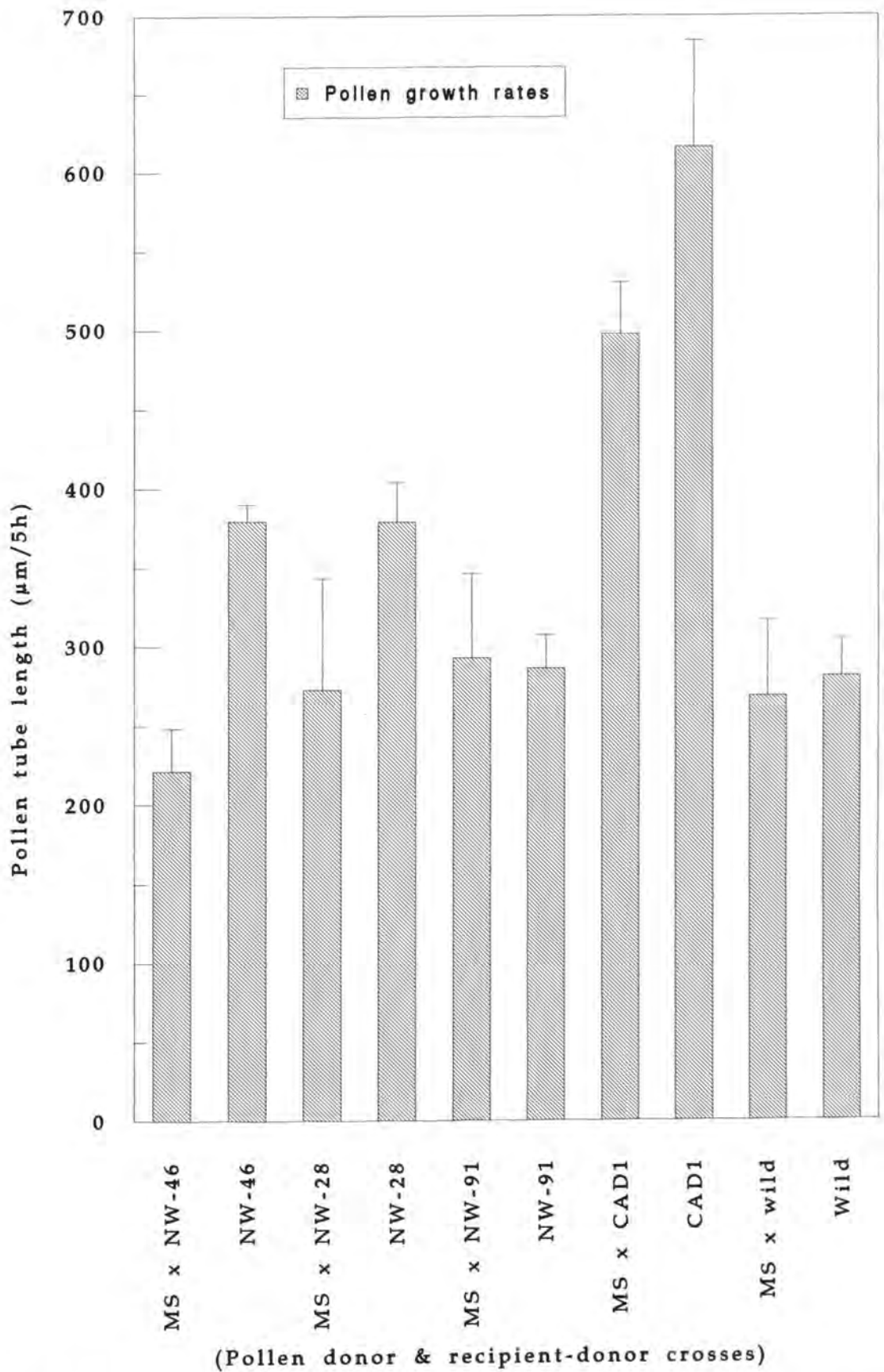
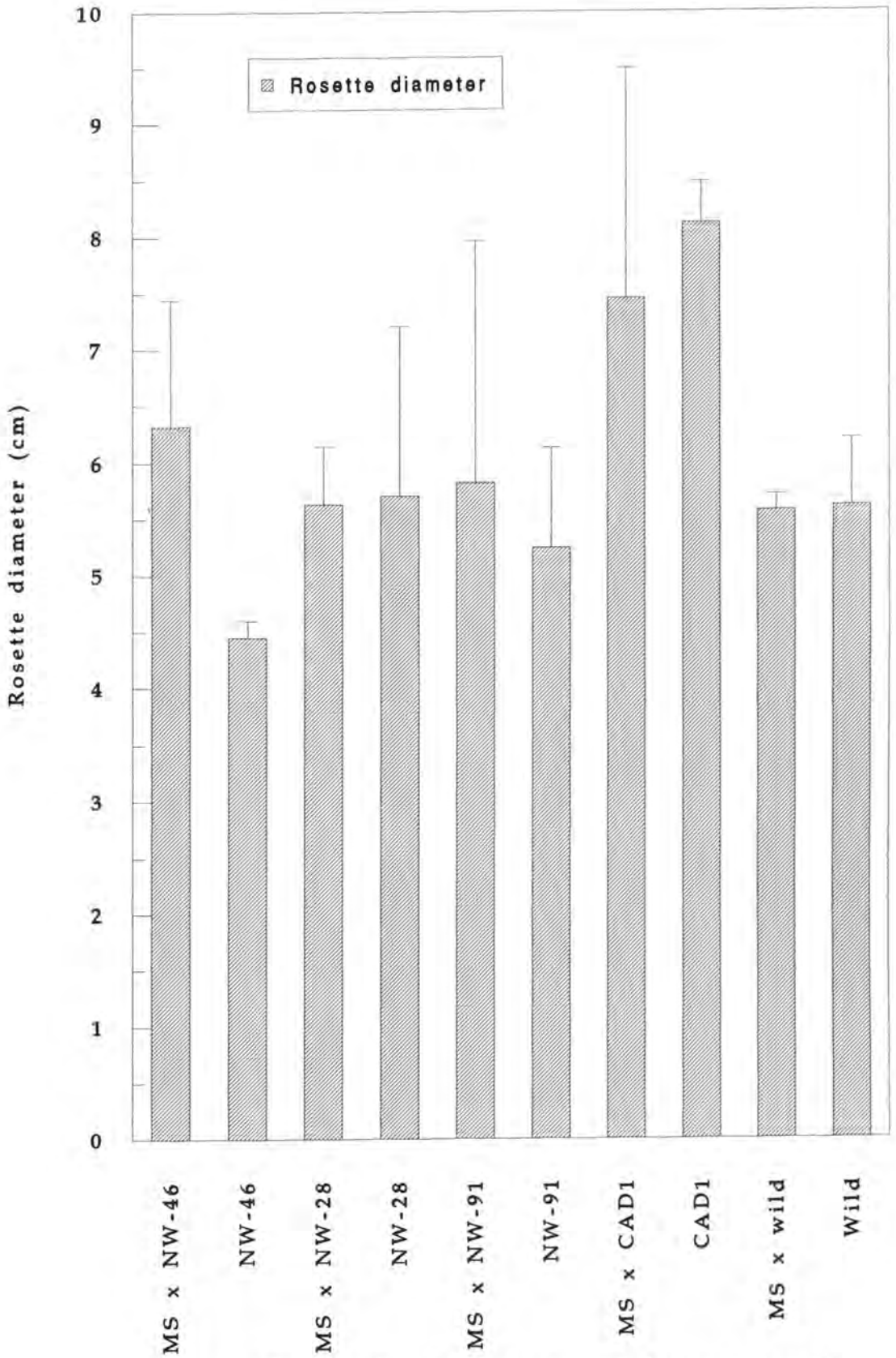
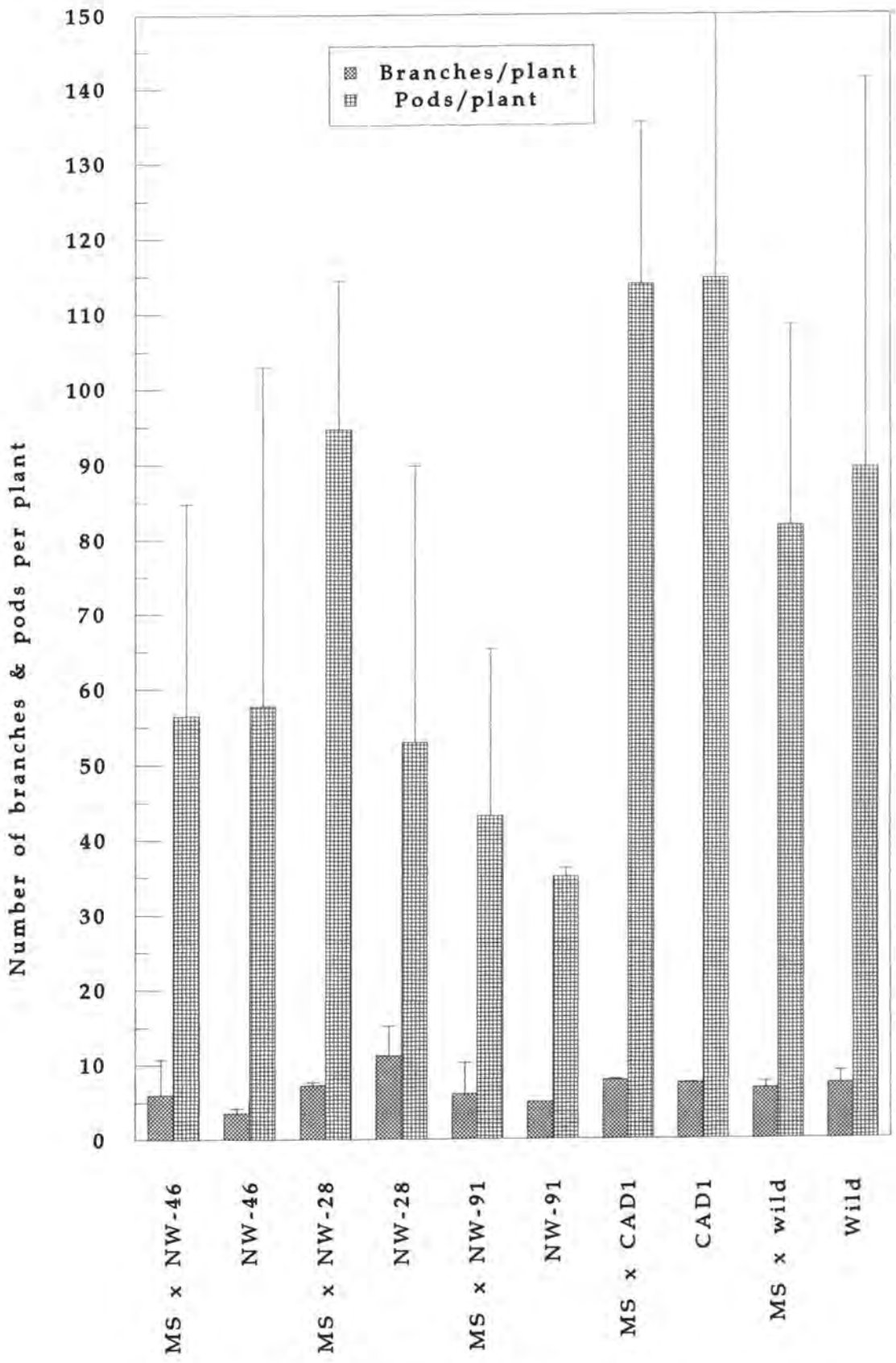


Fig. 5.3. Pollen growth rates ($\mu\text{m}/\text{h}$) for 5 hours incubation period of pollen donors and recipient-donor crosses in *A. thaliana*.



(Pollen donors & recipient-donor crosses)

Fig. 5.4. Rosette diameter (cm) of plants of pollen donors and recipient-donor crosses in *A. thaliana*.



(Pollen donors & recipient-donor crosses)

Fig. 5.5. Branches per plant & pods per plant of pollen donors and recipient-donor crosses in *A. thaliana*.

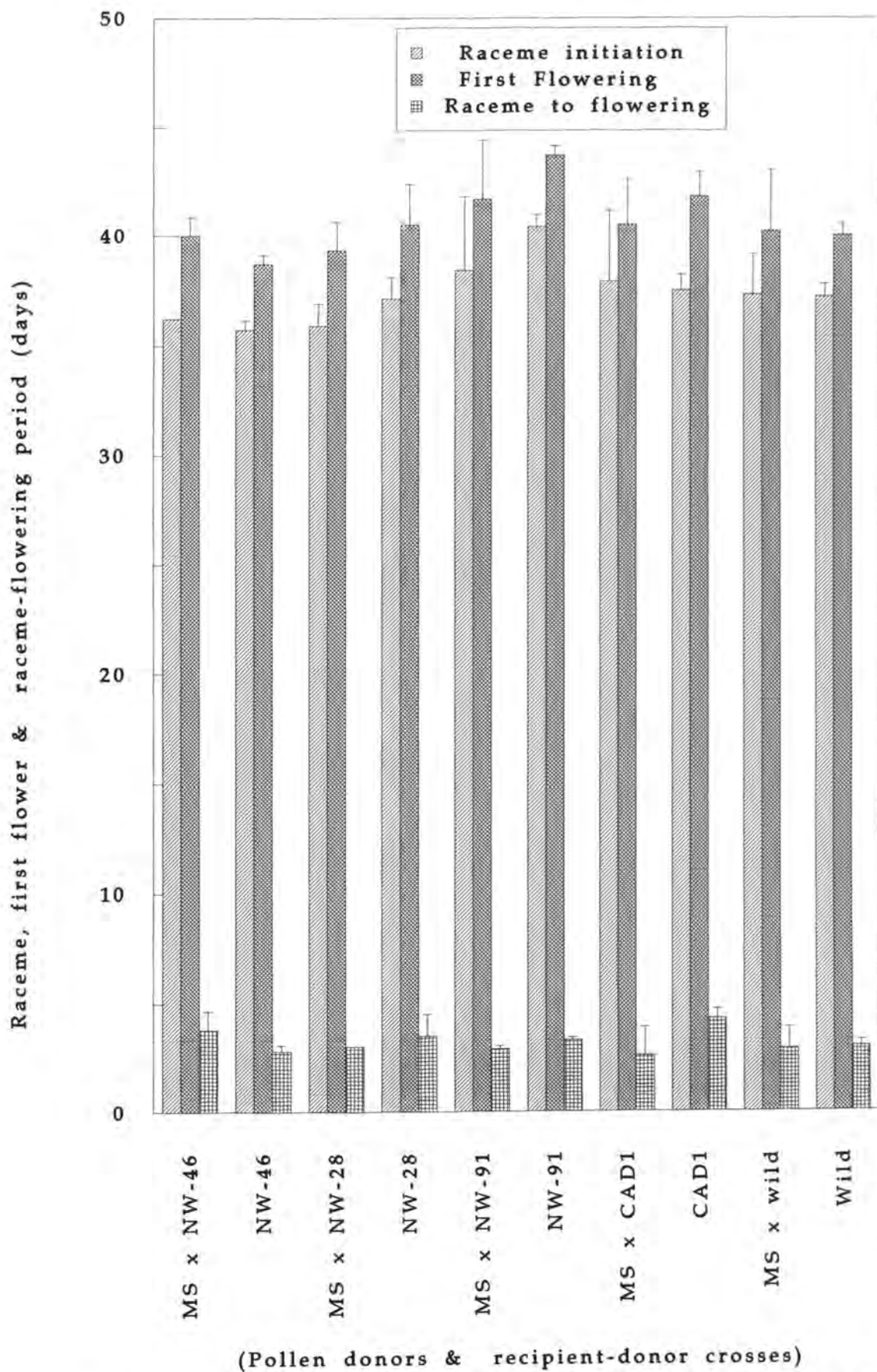


Fig. 5.6. Raceme, first flower initiation and period taken from raceme to flowering (days) for the pollen donors and recipient-donor crosses in *A. thaliana*.

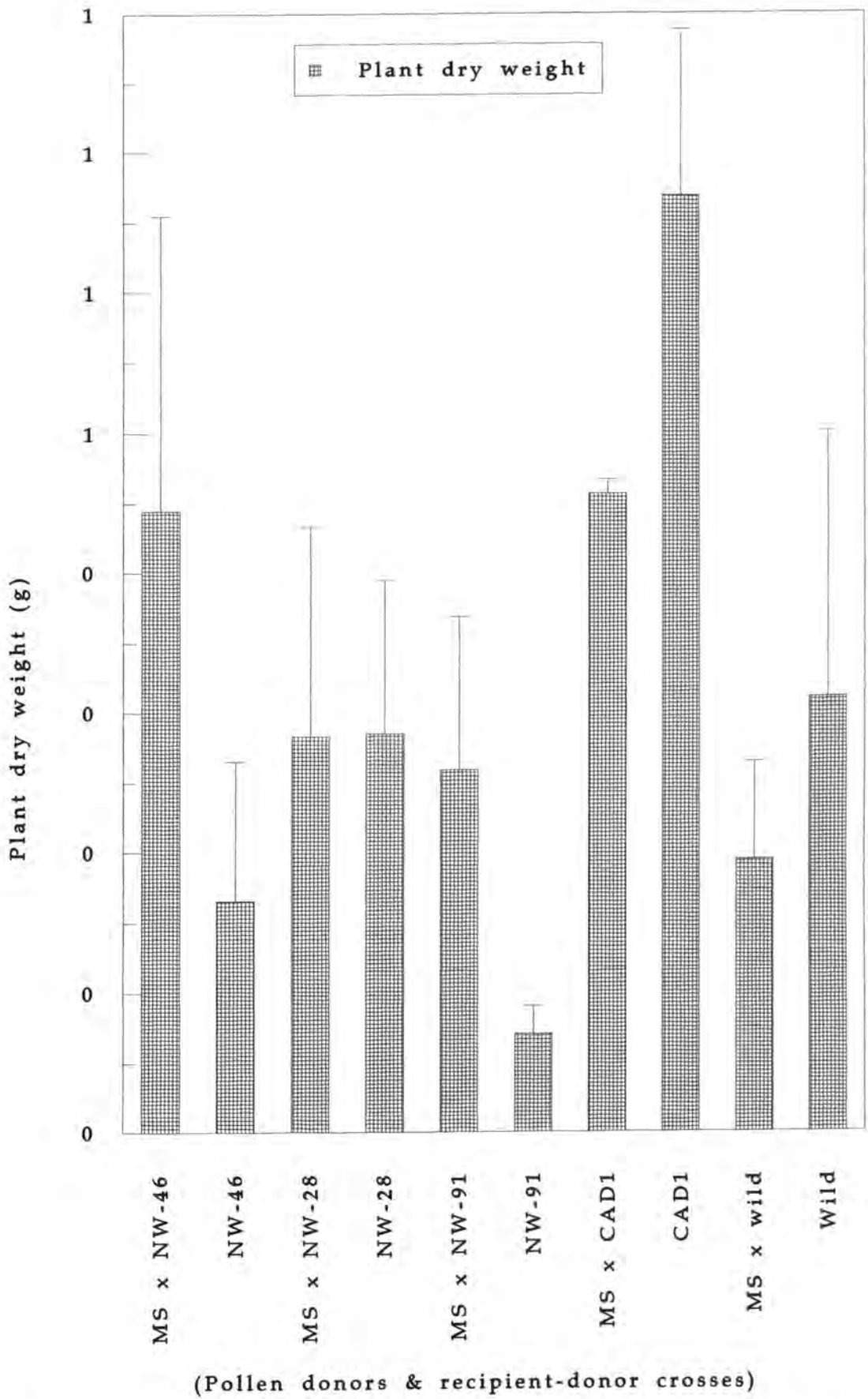


Fig. 5.7. Plant dry weight (g) of pollen donor plants and recipient-donor crosses in *A. thaliana*.

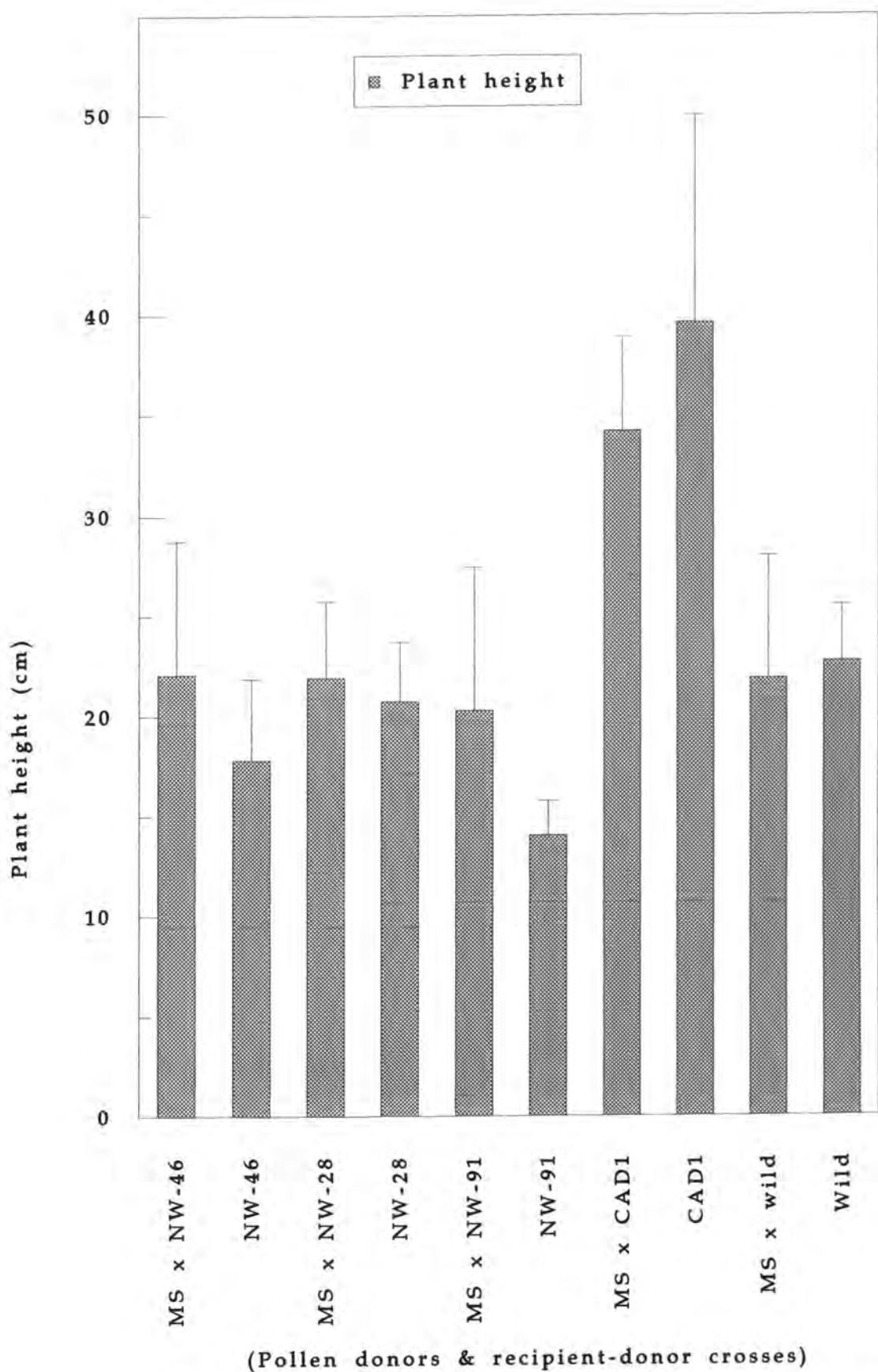


Fig. 5.8. Plant height (cm) of plants of pollen donors and recipient-donor crosses in *A. thaliana*.

Chapter VI

Effects of salinity (*NaCl*) on the sporophyte and gametophyte of

Arabidopsis thaliana

Experiment-1:

6.1 Male gametophyte: an index for prediction of salinity limits and selection for salt tolerance in plant species

While studying the consequences of the effect of salinity on pollen and the plant sporophyte in *Arabidopsis*, significant genotypic differences were observed in tolerance levels. The purpose of the following experiment was to determine whether exposure of the sporophyte to various levels of salinity would affect the gametophyte's level of tolerance.

6.1.1 Impact on the gametophyte

As already described in materials and methods, two groups of plants were studied to record the impacts of salinity. One group included plants raised in salinity stress-free conditions and another under a varying salinity-stress (SS) milieu. The pollen of the former group was subjected to the stress conditions by adding different concentrations of *NaCl* (100-2000 ppm) to the pollen germinating medium (PGM), while the pollen of the latter group was germinated in the stress-free PGM.

The experiment was designed to:

- (a). Test the effect of salinity stress on pollen germination and

pollen tube growth *in vitro* (stress-free plants).

(b). Test the effect of salinity stress of sporophytes on the subsequent pollen germination and growth of pollen forming in the anthers (salinity-stress plants).

Highly significant differences (Table-6.1) for pollen from stress-free plants with respect to pollen germination ($f = 459.72$, $df = 5$, $p \leq 0.001$), pollen tube length ($f = 202.30$, $df = 5$, $p \leq 0.001$), and non-significant differences ($f = 0.68$, $df = 2$) for pollen growth rates were recorded for added salinity levels against control (no added salinity). The pollen collected from salt-stressed plants (Table-6.2) also showed highly significant differences for PG ($f = 82.81$, $df = 6$, $p \leq 0.001$), PTL ($f = 12.97$, $df = 6$, $p \leq 0.001$) and PTGRs ($f = 28.07$, $df = 6$, $p \leq 0.001$) in comparison to the control.

Salt stress-free plants showed (Table-6.1, Fig. 6.1) a continuous decline in pollen germination percent (PG%) as increasing salinity stresses supplemented the PGM. The decline range varied from 13.97% (100 ppm) to 93.54 % (2000 ppm). The saline conditions stimulated the PTL from 19.35% to 38.31 % for added salinity levels of 100 and 500 ppm respectively, but inhibition of PTL was recorded when levels of *NaCl* were raised higher. The intensity of inhibition varied from 45.42% (1000 ppm) to 92.56 % (2000 ppm). The PTGRs could be determined only at two salinity levels viz 100 and 500 ppm, beyond which limit no pollen germination could be obtained during the 5 hr. incubation time which was fixed for evaluating the PTGRs. At both levels the PTGRs were stimulated (Fig. 6.3) from 5.37% (500 ppm) to 7.47 % (100 ppm) compared with control.

The salt-stressed plants (Table-6.2, Fig. 6.2) exhibited 0.48% (1000 ppm) to 51.42% (5000 ppm) inhibition, 1.91% (1000 ppm) to 24.17% (4000 ppm) and 3.79% (4000 ppm) and to 34.79 % (2000 ppm) stimulation respectively for PG, PTL and PTGRs compared with the control raised without saline regimes. Only in comparison with the 5000 ppm treatment (18.67%) did the control give higher PTGRs (Fig. 6.4).

6.1.2 Impacts of salinity on sporophytic stage

As mentioned in materials and methods, eight agronomic traits were taken in account to evaluate the influences of salinity on the sporophyte of *Arabidopsis* plants. Most of the characteristics showed highly significant responses towards salinity doses, while a few exhibited non-significant differences. The following are the details of the results obtained:

For clarity the sporophytic traits are sub-divided in three groups.

1. Effect of salinity on root
2. Effect of salinity on shoot
3. Effect of salinity on fruiting parts

6.1.2.1 Effect of salinity on root

Salinity levels significantly affected (Table-6.4) the plant root length ($f = 33.04$, $df = 6$, $p \leq 0.001$) and plant dry (Table-6.5) weight ($f = 11.05$, $df = 6$, $p \leq 0.001$).

Table-6.4 shows that there was a continuous decline from 4.48% (500 ppm) to 63.91 % (4000 ppm) in the root length. The RDW increased from 9.54 % (1000 ppm) to 14.31% (500 ppm) and decreased from 50.08% (2000 ppm) to 96.08%

(5000 ppm) at elevated salt levels.

6.1.2.2 Effect of salinity on shoot

Highly significant ($f = 46.35$, $df = 6$, $p \leq 0.001$) differences were obtained with respect to plant height between control and *NaCl* stressed plants. The height of plants was stimulated from 2.92% (2000 ppm) to 12.76% (1000 ppm). It then declined by 21.84% (3000 ppm) and by 59.95% (5000 ppm) against the control at the increasing levels of *NaCl* (Table-6.3) stress.

The data reveals (Table-6.3, Fig. 6.6) that non-significant ($f = 1.07$, $df = 6$) reduction of 4.77% (500 ppm) to 15.51% (5000 ppm) in the pedicel length occurred in comparison with control lengths.

Highly significant ($f = 48.84$, $df = 6$, $p \leq 0.001$) results were obtained for branches/plant. At the mild salinity levels the increases (Table-6.4, Fig. 6.8) over control varied from 0.92% (2000 ppm) to 22.32% (1000 ppm). A decline in branching ranging from 22.63% (3000 ppm) to 63.30% (5000 ppm) occurred at more elevated salt levels.

Table-6.5 and Fig. 6.9 indicate that at 500 ppm (180.38%) and 1000 ppm (41.28%) there was a non-significant ($f = 2.62$, $df = 6$) increase in comparison to the control in respect of plant shoot dry weight. The PSDW stimulated from 41.28% (1000 ppm) to 180.38% (500 ppm) under milder saline concentrations and decreased from 51.81% (2000 ppm) to 74.58% (4000 ppm) under elevated salinity levels in comparison to the control.

6.1.3 Effect of salinity on fruiting parts

The pod length varied significantly ($f = 10.48$, $df = 6$, $p \leq 0.001$) for the different doses of salinity applied. It was stimulated (Table-6.3, Fig. 6.6) relative to the control from 1.59% (500 ppm) to 4.41% (1000 ppm) and decreased at elevated levels of *NaCl* from 0.98% (2000 ppm) to 12.62% (5000 ppm) of the control level.

The data (Table-6.4, Fig. 6.7) show that an *NaCl* concentration of 1000 ppm (5.41%) significantly ($f = 81.02$, $df = 6$, $p \leq 0.001$) produced more pods/plant in relation to the control. Under the remaining concentrations fewer pods/plant, varying from 6.71% (500 ppm) to 96.54 (5000 ppm) of the control, were obtained.

Experiment-2

6.2 Fruit-setting, seed-setting and seed-filling in *A. thaliana*

in response to high salinity (*NaCl*) conditions

As described in materials and methods, the pollen donors and pollen recipients were raised under different salinity regimes. After pollination, the successful rate of fertilization, number of seeds/pod and filled seed percentage were recorded. The pollen germination percentage and pollen tube length from pollen donors, and plant fresh weight and plant dry weight were also noted.

6.2.1 Pollen germination and growth

Statistically nonsignificant differences for pollen germination ($f = 2.68$, $df = 1$, $p \leq 0.140$) and pollen tube length ($f = 1.83$, $df = 1$, $p \leq 0.213$) were recorded for the pollen collected from the plants grown under high salinity (5000 ppm) regimes and salinity stress-free conditions.

Germinating the pollen from both plant sources i.e from high salinity stressed and stress-free plants with 1000 ppm added salinity (intermediate salinity level) in the pollen germinating medium; nonsignificant differences were observed between both plant groups. The arithmetical increase of 41.50% and 27.44% was recorded respectively for PG and PTL in the double-stressed pollen over the single-stressed pollen (Table-6.6).

6.2.2 Effect on fruit-setting

As stated in materials and methods the term HS (high stress) has been used for plants grown under the salinity stress of 5000 ppm, and the term SF (stress

free) has been used for plants grown without salinity stress.

The highest fruit-setting percentage of crosses was noted (Table-6.7) for the control combination MS-SF x wild-SF (92.10%), while the least percentage was recorded for MS-SF x wild-HS (81.35%). Comparing this with the cross having highest fruiting levels (MS-SF x wild-SF), declines of 8.13%, 11.37% and 11.67% were observed in the hybrid combinations MS-HS x wild-HS, MS-HS x wild-SF and MS-SF x wild-HS, respectively.

6.2.3 Effect on seed-setting

Highly significant differences ($f = 9.07$, $df = 5$, $p \leq 0.00$) for seeds set/pod were noted for the plants raised in different salinity regimes. Compared with control (wild-SF), declines (Table-6.7) of 8.63% (MS-SF x wild SF) to 52.27% (MS-SF x wild-HS) were observed.

6.2.4 Effect on seed-filling

For percent filled seed highly significant differences ($f = 5.24$, $df = 5$, $p \leq 0.002$) were noted. Compared with the control (wild-SF), an increase of 5.10% was observed in (Table-6.7) the combination MS-SF x wild-SF. Declines of 1.05%, 7.18% and 14.21% were observed in MS-HS x wild-HS, MS-HS x wild-SF and MS-SF x wild-HS, respectively.

6.2.5 Plant fresh and dry weight

Because of the limited number of plants no statistical analysis could be performed. Comparing arithmetic means (Table-6.8), a decrease of 53.42% and 48.30% was observed in MS-HS plants against MS-SF respectively for fresh and dry plant

weight. In the case of the wild genotype, the decline of 35.84% and 32.98% was noted in treatment wild-HS against wild-SF for plant fresh and dry plant weight respectively.

Table-6.1: PG, PTL and PTGRs of the *Arabidopsis thaliana* (wild type), pollen, collected from **salinity stress-free plants**, and tested in **saline pollen germinating media**.

<i>NaCl</i> concen.	PG (%)	% inc. (+) or dec. (-)	PTL (μm)	% inc. (+) or dec. (-)	PTGRs ($\mu\text{m}/5\text{h}$)	% inc. (+) or dec. (-)
100 ppm	61.46 (2.90)	-13.97 -	656.63 (19.00)	+19.35 -	213.20 (15.69)	+7.47 -
500 ppm	42.57 (2.67)	-40.41 -	760.93 (37.45)	+38.31 -	209.03 (25.00)	+5.37 -
1000 ppm	19.49 (1.89)	-72.72 -	300.29 (41.49)	-45.42 -	nd -	- -
1500 ppm	11.32 (0.80)	-84.15 -	204.96 (83.24)	-62.74 -	nd -	- -
2000 ppm	4.61 (1.17)	-93.54 -	40.92 (1.67)	-92.56 -	nd -	- -
Control	71.44 (3.82)	- -	550.15 (26.87)	- -	198.38 -	- -
F (df)	459.72 (5)	-	202.3 (5)	-	0.68 (2)	-
P \leq	0.001	-	0.001	-	ns	-

SD are in parenthesis

nd = means not determined

Table-6.2: PG, PTL and PTGRs of the *Arabidopsis thaliana* (wild type), pollen collected from **salinity stressed plants**, and tested under **nonsaline pollen germinating medium**.

<i>NaCl</i> concent.	PG (%)	% inc. (+) or dec. (-)	PTL (μm)	% inc. (+) or dec. (-)	PTGRs (μm)	% inc. (+) or dec. (-)
500 ppm	69.49 (4.93)	-1.96 -	728.66 (48.73)	+17.35 -	312.92 (15.63)	+16.65 -
1000 ppm	70.54 (3.30)	-0.48 -	732.45 (96.79)	+17.96 -	359.52 (28.52)	+34.02 -
2000 ppm	48.20 (3.85)	-31.99 -	768.74 (27.34)	+23.80 -	361.60 (13.45)	+34.79 -
3000 ppm	44.91 (3.19)	-36.64 -	707.54 (58.39)	+13.95 -	344.04 (29.01)	+28.25 -
4000 ppm	38.72 (4.68)	-45.37 -	771.01 (18.94)	+24.17 -	278.42 (13.10)	+3.79 -
5000 ppm	34.43 (1.26)	-51.42 -	632.83 (19.22)	+1.91 -	218.17 (21.38)	-18.67 -
Control (MSS)	70.88 (1.61)	- -	620.94 (32.45)	- -	268.26 (3.55)	- -
F (df)	82.81 (6)		12.97 (6)	-	28.07 (6)	
P \leq	0.001	-	0.001	-	0.001	

SD are in parenthesis

Table-6.3: Impact of different levels of salinity stresses (*NaCl*), on the sporophytic traits of *Arabidopsis thaliana* (wild type)

<i>NaCl</i> concent.	Plant height (cm)	% inc. (+) or dec. (-)	Pedicel length (mm)	% inc. (+) or dec. (-)	Pod length (mm)	% inc. (+) or dec. (-)
500 ppm	20.30 (0.81)	+7.98 -	3.99 (0.33)	-4.77 -	8.29 (0.41)	+1.59 -
1000 ppm	21.20 (1.22)	+12.76 -	3.99 (0.45)	-4.77 -	8.52 (0.52)	+4.41 -
2000 ppm	19.35 (0.85)	+2.92 -	3.66 (0.62)	-12.65 -	8.08 (0.59)	-0.98 -
3000 ppm	14.73 (1.66)	-21.84 -	3.85 0.54	-8.11 -	7.89 (0.11)	-3.31 -
4000 ppm	9.63 (1.40)	-48.78 -	3.81 (0.11)	-9.07 -	7.42 (0.18)	-9.06 -
5000 ppm	7.53 (1.80)	-59.95 -	3.54 (0.42)	-15.51 -	7.13 (0.17)	-12.62 -
Control (MSS)	18.80 (1.13)	- -	4.19 (0.21)	- -	8.16 (0.35)	- -
F (df)	46.35 (6)	-	1.07 (6)	-	10.48 (6)	-
P ≤	0.001	-	ns	-	0.001	-

SD are in parenthesis

Table-6.4: Impact of different levels of salinity stresses (*NaCl*) on sporophytic traits of *Arabidopsis thaliana* (wild type)

<i>NaCl</i> concent.	Branches per plant	% inc. (+) or dec. (-)	Pods per plant	% increase (+) or dec. (-)	Root length (cm)	% inc. (+) or dec. (-)
500 ppm	3.85 (0.07)	+17.73 -	35.87 (5.11)	-6.71 -	12.36 (1.50)	-4.48 -
1000 ppm	4.0 (0.40)	+22.32 -	40.53 (1.01)	+5.40 -	10.33 (1.92)	-20.17 -
2000 ppm	3.30 (0.05)	+0.92 -	29.65 (2.45)	-22.89 -	10.65 (1.15)	-17.70 -
3000 ppm	2.53 (0.42)	-22.63 -	26.86 (6.09)	-30.14 -	7.97 (0.57)	-38.40 -
4000 ppm	1.53 (0.42)	-53.21 -	4.27 (1.17)	-88.89 -	4.67 (1.16)	-63.91 -
5000 ppm	1.20 (0.35)	-63.30 -	1.33 (0.30)	-96.54 -	8.83 (1.10)	-31.76 -
Control (MSS)	3.27 (0.11)	- -	38.45 (1.72)	- -	12.94 (2.21)	- -
F (df)	48.84 (6)	-	81.02 (6)	-	33.04 (6)	-
P ≤	0.001	-	0.001	-	0.001	-

SD are in parenthesis

Table-6.5: Impact of different levels of salinity stresses (*NaCl*) on sporophytic traits of *Arabidopsis thaliana* (wild type)

<i>NaCl</i> concent.	Root dry wt. (g)	% inc. (+) or dec. (-)	Shoot dry wt. (g)	% inc. (+) or dec. (-)
500 ppm	0.00671 (0.00383)	+14.31 -	0.11294 (0.10402)	+180.38 -
1000 ppm	0.00643 (0.00173)	+9.54 -	0.05691 (0.01757)	+41.28 -
2000 ppm	0.00293 (0.00211)	-50.08 -	0.01941 (0.00435)	-51.81 -
3000 ppm	0.00292 (0.00156)	-50.25 -	0.01514 (0.00671)	-62.41 -
4000 ppm	0.00023 (0.00041)	-83.98 -	0.01024 (0.00198)	-74.58 -
5000 ppm	0.00023 (0.00004)	-96.08 -	0.01151 (0.00204)	-71.42 -
Control (MSS)	0.00587 (0.0010)	- -	0.04028 (0.01368)	- -
F (df)	11.05 (6)	-	2.62 (6)	-
P ≤	0.001	-	ns	-

SD are in parenthesis

Table-6.6: Performance of *Arabidopsis thaliana* (wild type) pollen under **single-stress** (pollen collected from stress-free (SF) plants and germinated in the PGM supplemented with 1000 ppm, *NaCl*) and **double-stress** salinity conditions (pollen collected from plants raised under 5000 ppm *NaCl* (HS) and germinated in the PGM added with 1000 ppm *NaCl*).

Plant group	PG (%)	% inc. (+) or dec. (-)	PTL (μm)	% inc. (+) or dec. (-)
Wild-HS (5000 ppm)	36.55 (11.41)	+41.50 -	429.02 (113.52)	+27.44 -
Wild-SF (MSS)	25.83 (9.14)	- -	336.64 (101.71)	- -
F (df)	2.68 (1)	-	1.83 (1)	-
P \leq	0.40	-	0.213	-

SD are in parenthesis

Table-6.7: Effects of high salinity concentration (5000 ppm) on the fruit-setting, seed-setting and seed-filling in *Arabidopsis thaliana*.

Combination	Crosses attempted	Succ. cross (%)	% inc.(+) or dec.(-)	Seed setting per pod	% inc. (+) or dec.(-)	Filled seed (%)	% inc.(+) or dec. (-)
MS-HS x wild-HS	65	84.61	-8.13	29.20 (6.90)	-33.64	87.52 (6.67)	-1.05
MS-HS x wild-SF	49	81.83	-11.37	20.20 (6.16)	-54.09	82.21 (8.23)	-7.18
MS-SF x wild-HS	59	81.35	-11.67	21.00 (2.34)	-52.27	75.98 (7.99)	-14.21
MS-SF x wild-SF	76	92.10	-	40.20 (7.19)	-8.63	93.09 (6.04)	+5.10
Wild-HS	-	-	-	28.60 (9.07)	-35.00	78.59 (3.85)	-11.27
Wild-SF (control)	-	-	-	44.00 (2.64)	-	88.57 (3.91)	-
F (df)	-	-	-	9.07 (5)	-	5.24 (5)	-
P ≤	-	-	-	0.00	-	0.002	-

SD are in parenthesis

Table-6.8: Effects of high salinity concentrations (5000 ppm) on plant fresh and dry weight in *Arabidopsis*

Plant group	FPW (g)	% inc. (+) or dec. (-)	DPW (g)	% inc. (+) or dec.
MS-HS	0.9252	-53.42	0.5162	-48.30
MS-SF	1.9862	-	0.9986	-
Wild-HS	1.3854	-35.84	0.7582	-32.98
Wild-SF	2.1593	-	1.1314	-

6.3 Anatomical changes brought by salinity

Increasing concentrations of *NaCl* produced the following general changes in the anatomy of the stem, leaf and root tissues.

6.3.1 Effect of salinity on stem anatomy

The following changes were recorded in the stem tissues (Plates-6.1-6.4) of the seedlings treated with different concentrations of *NaCl*.

- (a). The epidermal cells were larger than the control.
- (b). The cortical cells were larger and thinner, with more cortical layers at higher salt concentrations.
- (c). The pericycle was more lignified than the control.
- (d). The xylem was more lignified than the control at elevated saline conditions.
- (e). The pith cells were larger than the control.
- (f). The cross sectional diameter of the vascular bundle (CSDVB) was reduced compared with the control.

Summary of measurements

While quantifying the changes in the stem tissues (Table-6.9), it was observed that the diameters of epidermal cell declined at lower salinity levels and increased under increasing saline levels in relation to the control. The cortex cell size was stimulated in elevated saline concentrations. The pith cell diameter decreased below 500 ppm *NaCl* and increased under other salinity stress regimes. The CSDVB declined at increasing salinity levels.

6.3.2 Effect of salinity on leaf anatomy

The salinity treatments created (Plates-6.5-6.6) the following changes in the leaf tissues:

- (a). The reduction in the cell size of the the adaxial and abaxial epidermis in the treated seedlings.
- (b). An increase in the palisade cell size compared with the control.
- (c). A reduction in the size of the intercellular spaces compared with the control.

The data (Table-6.10) indicate that the adaxial epidermal cell size decreased under every saline stress condition and the abaxial epidermal cell size was stimulated under the lowest saline concentration and decreased in higher saline conditions. The palisade parenchyma cell diameter declined under the lowest salinity concentration and increased at elevated stress conditions. The intercellular spaces decreased in size compared with the control under all saline-stress states.

6.3.3 Effect of salinity on root anatomy

Following anatomical changes were observed (Plate-6.7-6.8) in the root tissues of the seedlings treated with different concentrations of salinity:

- (a). The cortex cells were larger than the control.
- (b). A lignified and undistinguishable pericycle and phloem was observed.
- (c). Larger xylem cells developed.
- (d). Larger stele size diameter.

The cortex cell size was stimulated (Table-6.11) under lower saline levels in

comparison to the control and was affected adversely under highest saline-stress conditions. The stele diameter increased under all salinity-stress regimes.

Table-6.9 : The diameter of epidermal, cortex and pith cells & cross sectional diameter of vascular bundle (CSDVB) (μm) of stem of *A. thaliana* raised under different saline (*NaCl*) regimes.

Tissue/cell	<i>NaCl</i> (ppm)			Control
	250	500	1000	
Epidermis	10.89±1.33 (-13.57)	14.60±1.85 (+15.87)	18.10±4.18 (+43.67)	12.60±2.46 -
Cortex	17.60±5.46 (+8.75)	17.10±2.98 (+6.87)	17.80±3.15 (+11.25)	16.00±1.79 -
Pith	53.33±9.91 (+15.93)	24.80±1.93 (-46.08)	54.00±12.58 (+17.39)	46.00±10.11 -
CSDVB	74.00±10.00 (-53.75)	121.13±10.00 (-24.29)	121.00±12.77 (-24.37)	160.00±3.26 -

Table-6.10: The diameter of adaxial and abaxial epidermal cells, palisade parenchyma cells and size of intercellular spaces (μm) of leaves of *A. thaliana* raised under different saline (*NaCl*) regimes.

Tissue/cell	<i>NaCl</i> (ppm)			Control
	250	500	1000	
Adaxial epidermis	35.71 \pm 2.22 (-37.13)	41.43 \pm 5.59 (-27.23)	43.43 \pm 11.45 (-16.50)	56.80 \pm 8.68 -
Abaxial epidermis	38.67 \pm 5.22 (+5.94)	33.20 \pm 3.72 (-9.04)	35.60 \pm 5.42 (-2.46)	36.50 \pm 1.41 -
Palisade parenchyma	39.00 \pm 9.52 (-7.14)	50.00 \pm 4.73 (+19.04)	49.00 \pm 10.59 (+16.67)	42.00 \pm 10.77 -
Intercellular spaces	62.00 \pm 12.33 (-25.59)	26.80 \pm 4.83 (-67.84)	39.33 \pm 10.87 (-52.80)	83.33 \pm 13.89

Table-6.11: The diameter of stele & size of cortex cells (μm) of the root of *A. thaliana* raised under different saline-stress (*NaCl*) regimes.

Tissue/cell	<i>NaCl</i> (ppm)			Control
	250	500	1000	
Cortex	22.75 \pm 2.38 (+82.00)	22.50 \pm 6.24 (+80.00)	ndv -	12.50 \pm 3.64 -
Stele	37.00 \pm 3.00 (+2.78)	45.00 \pm 15.00 (+45.00)	80.00 \pm 10.00 (+122.22)	36.00 \pm 1.00 -

ndv denotes nondetermined values

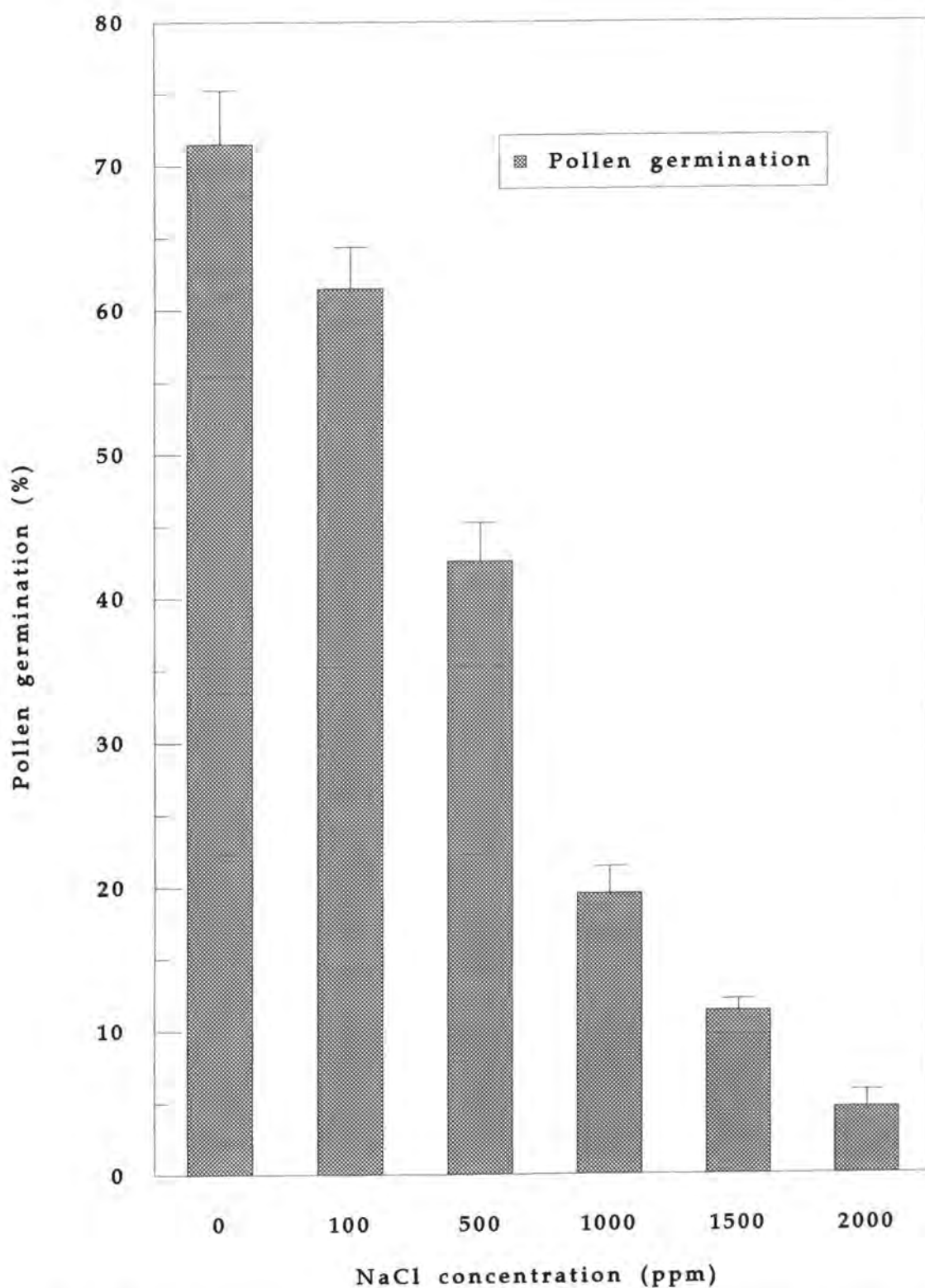


Fig.6.1. Pollen germination (%) of *A. thaliana* (wild type), pollen, collected from salinity stress-free plants and germinated in pollen media supplemented with different saline (NaCl) levels.

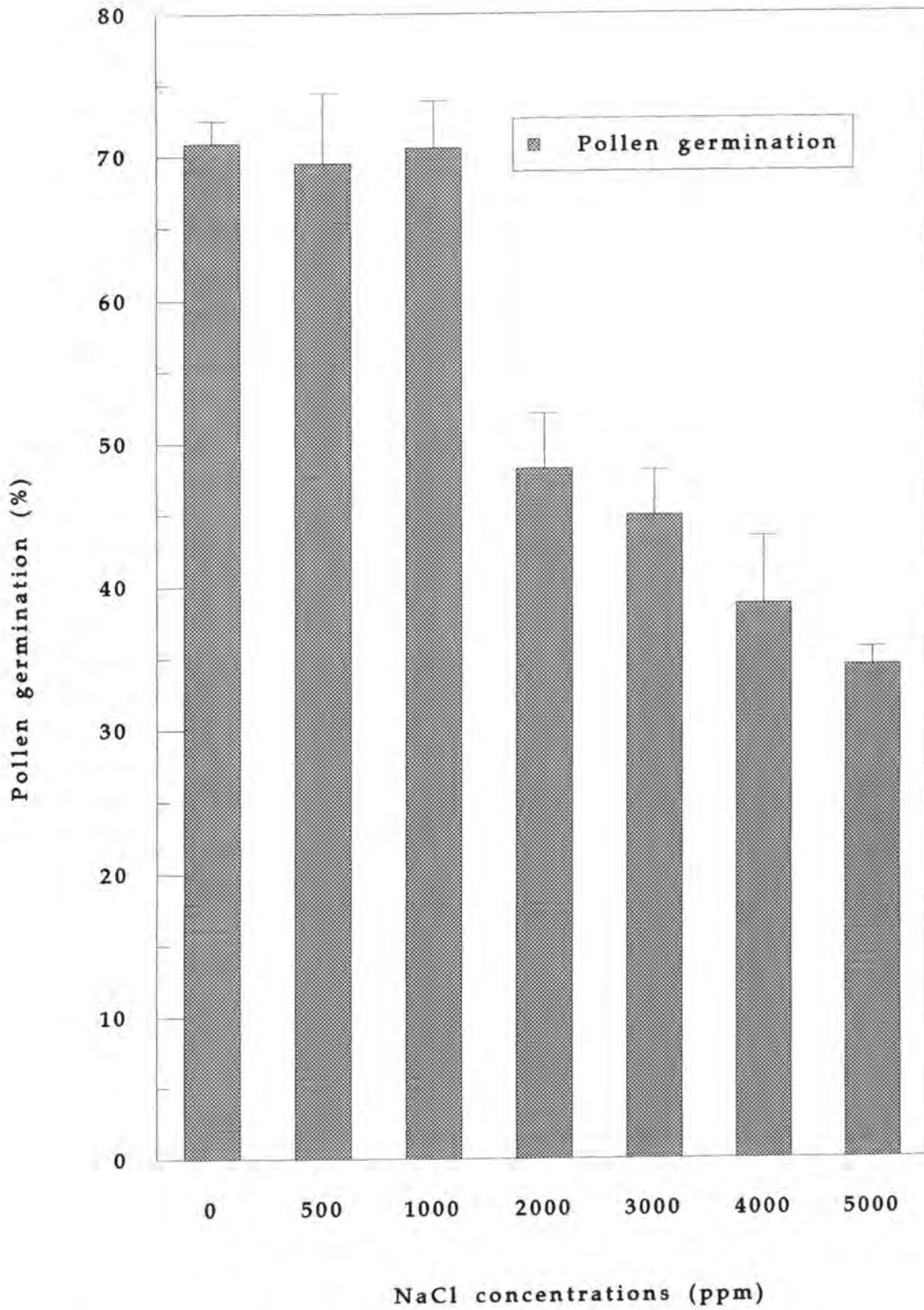


Fig.6.2. Pollen germination (%) of *A. thaliana* pollen, collected from saline-stressed (NaCl) plants and germinated in non-saline pollen medium.

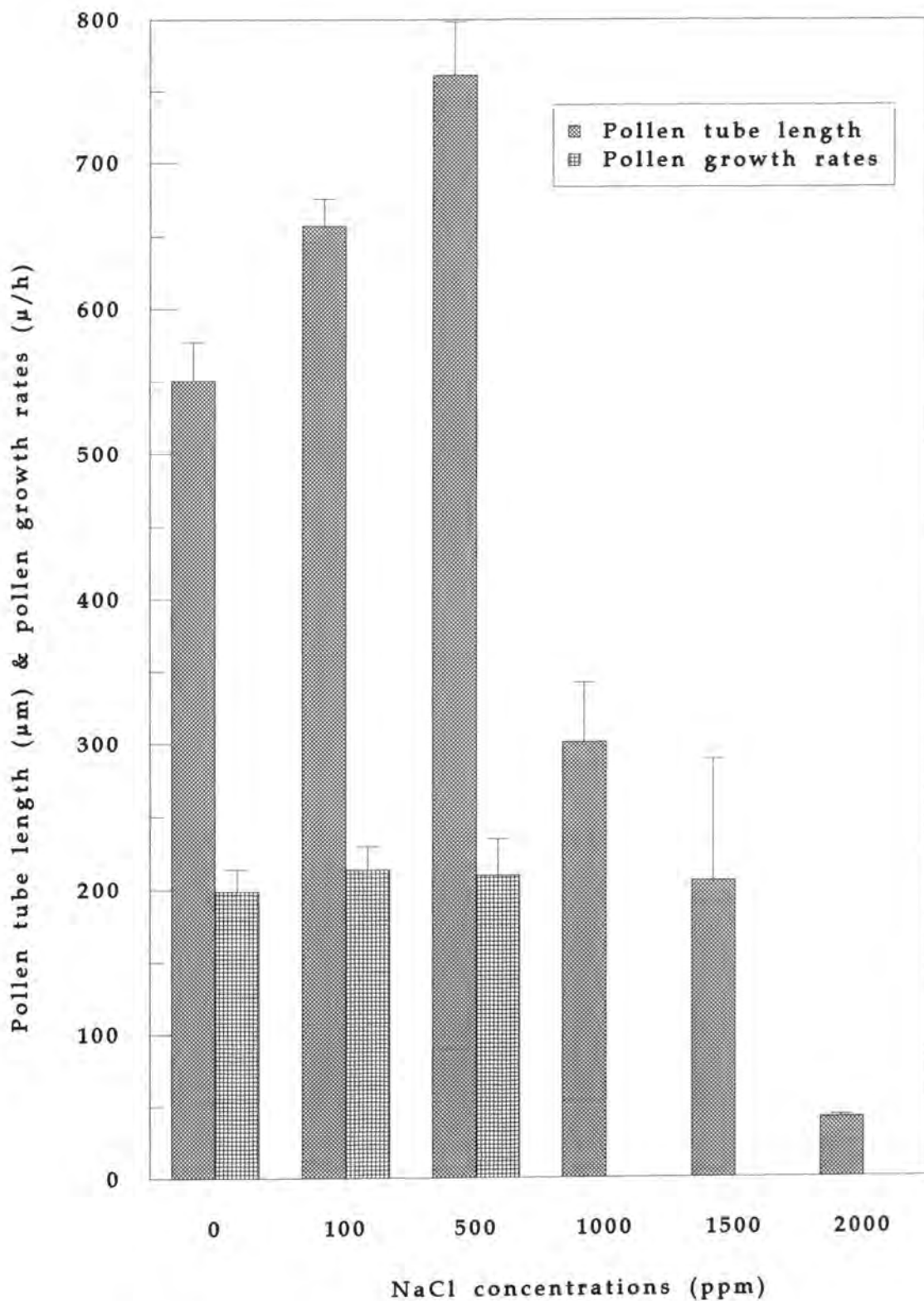


Fig.-6.3 Pollen tube length (μm) and pollen tube growth rates ($\mu\text{m}/5\text{h}$) of the *A. thaliana* pollen, collected from salinity stress-free plants and germinated in saline pollen media supplemented with different added salinity (NaCl) levels.

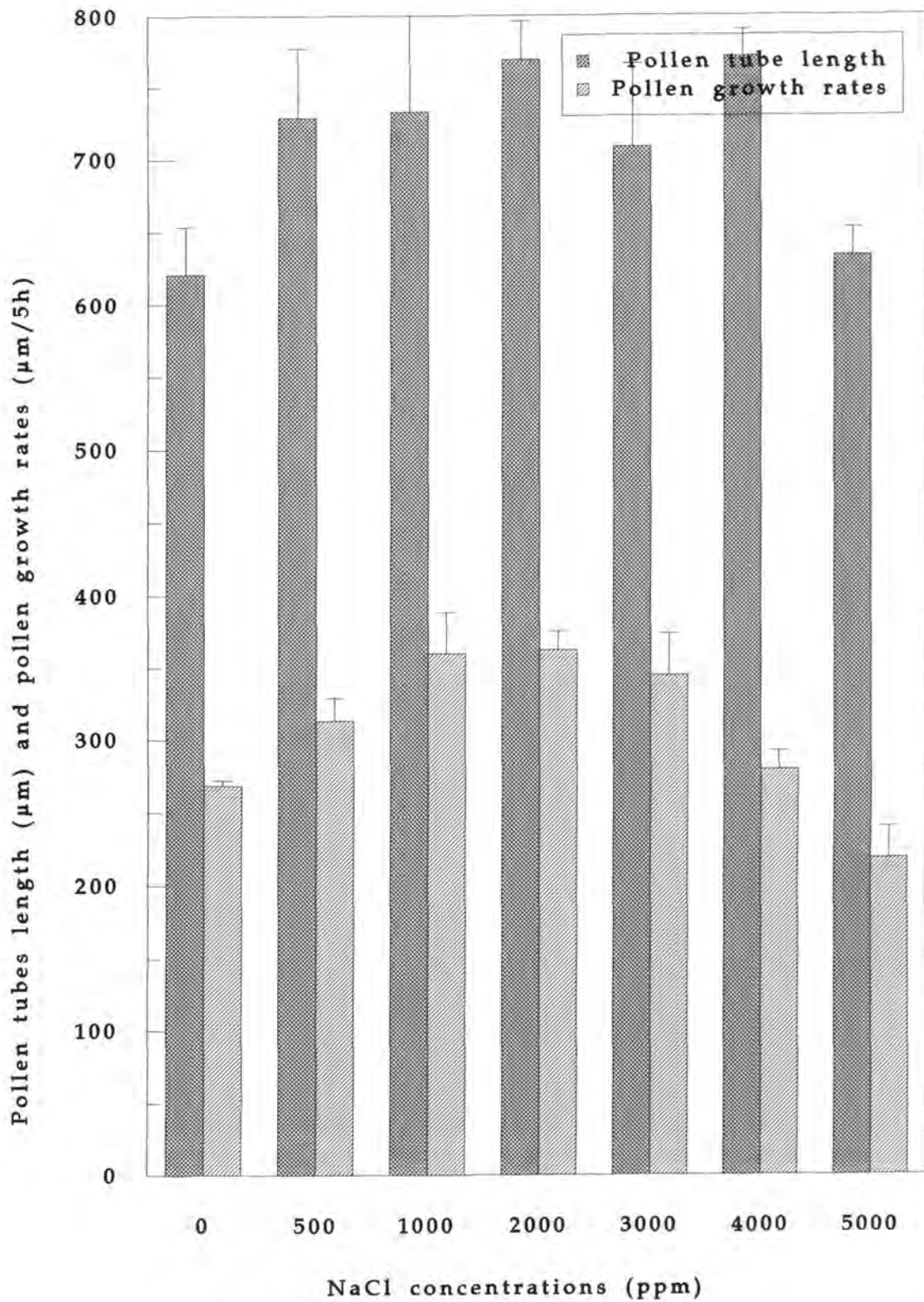


Fig.6.4 Pollen tube length (μm) & pollen growth rates ($\mu\text{m}/5\text{h}$) of *A. thaliana* (wild type), pollen, collected from salinity-stressed (NaCl) plants and germinated in non-saline pollen medium.

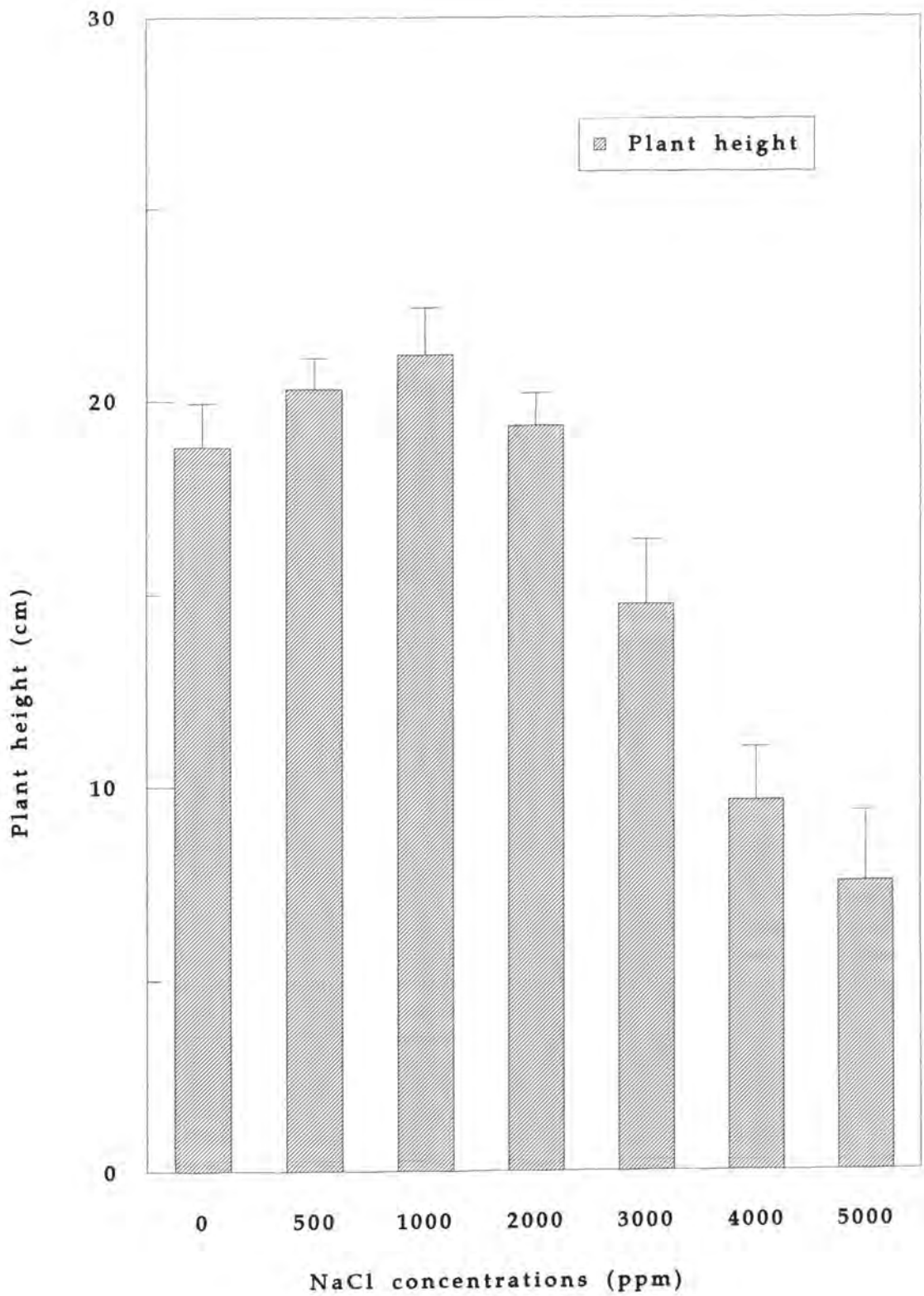


Fig.6.5. Plant height (cm) of *A. thaliana* (wild type) plants raised under different saline (NaCl) stress conditions.

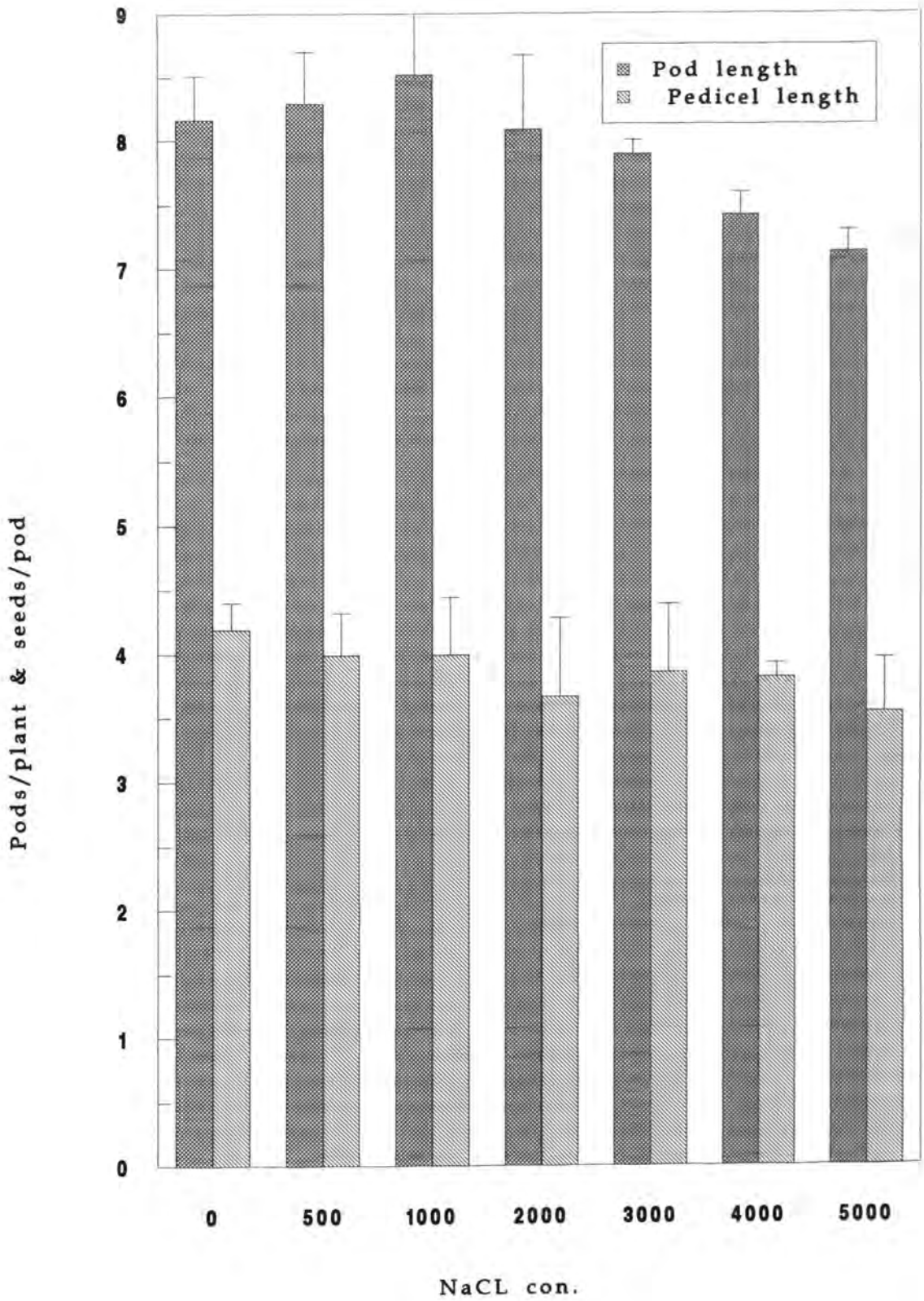


Fig. 6.6. Pod and pedicel length (mm) of *A. thaliana* (wild type), plants, raised under different saline (NaCl) stress-conditions.

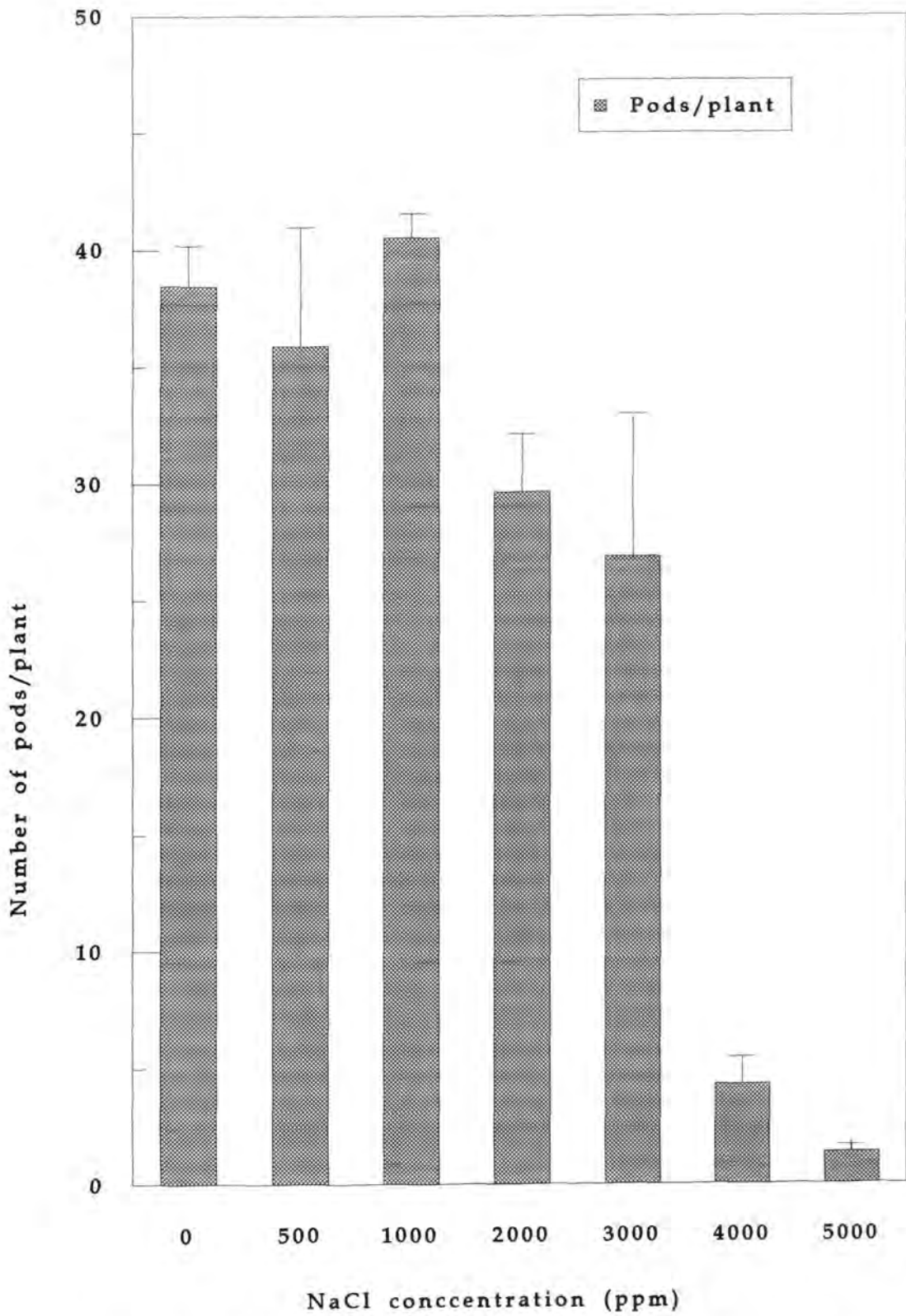


Fig. 6.7. Pods per plant in *A.thaliana* (wild type), plants, raised under different saline (NaCl) stress conditions.

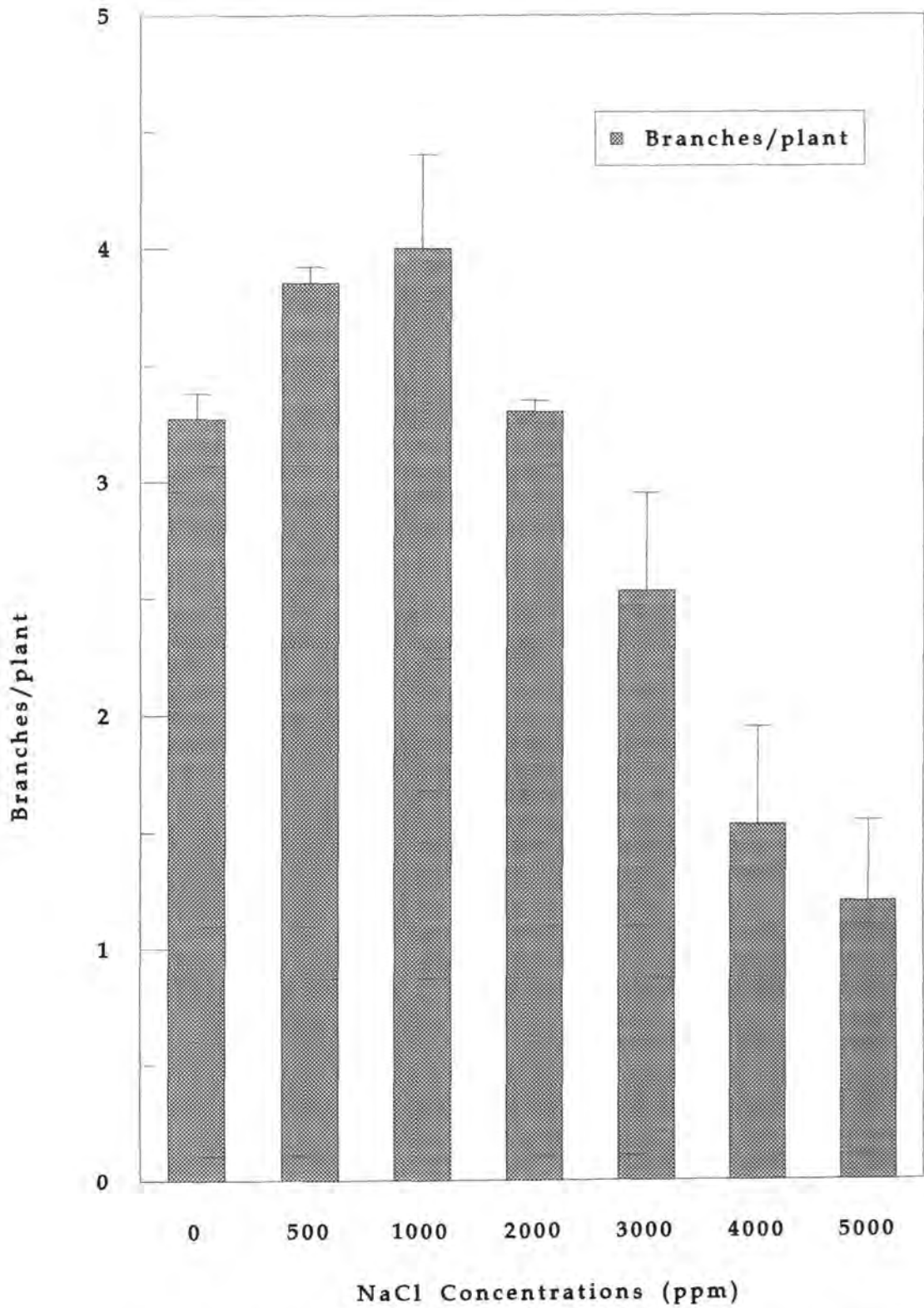


Fig.6.8. Branches per plant in *A. thaliana* (wild type), plants, raised under different saline (NaCl) stress conditions.

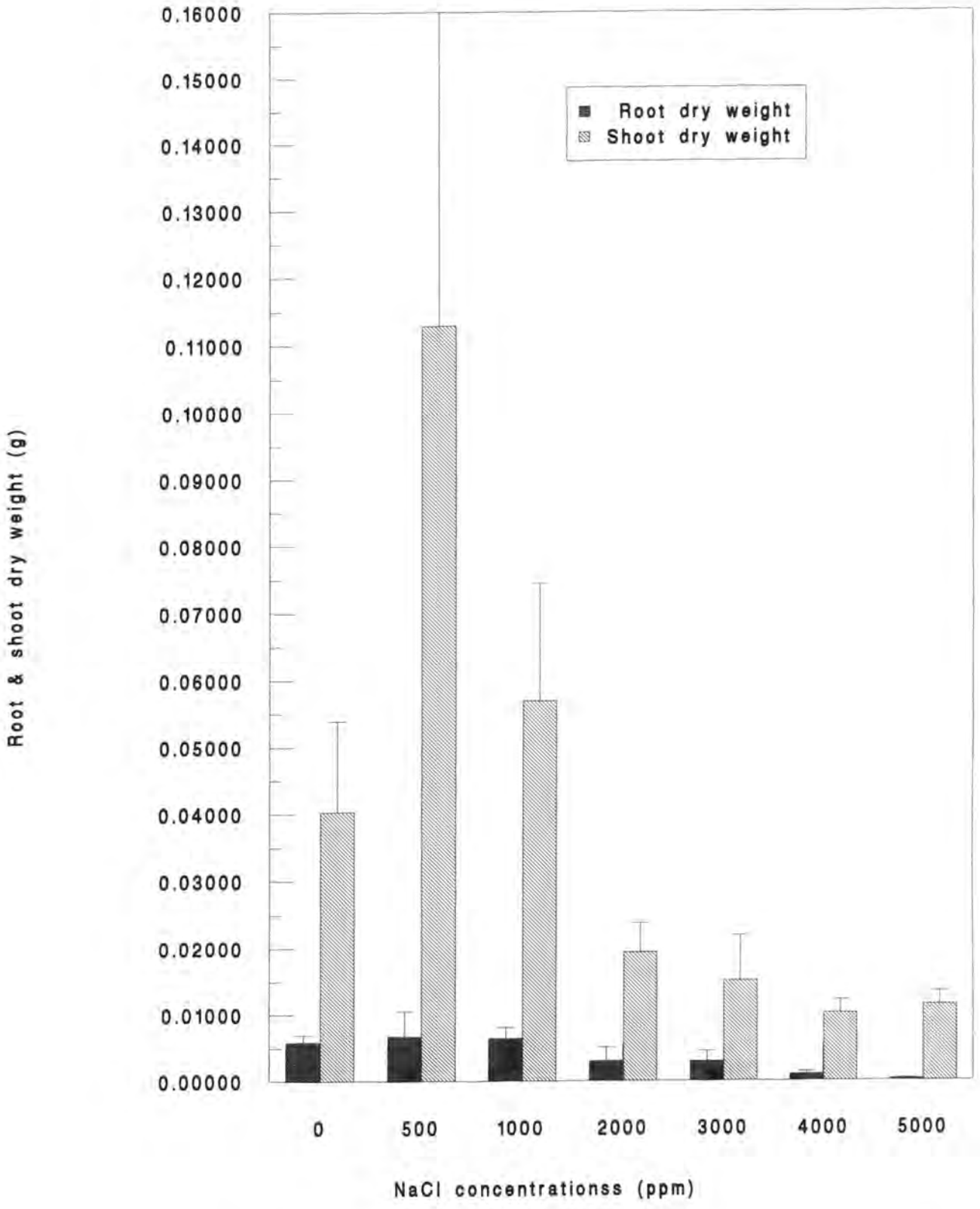


Fig. 6.9. Root and shoot dry weight (g) of *A. thaliana* (WT), plants raised under different saline (NaCl) stress conditions.

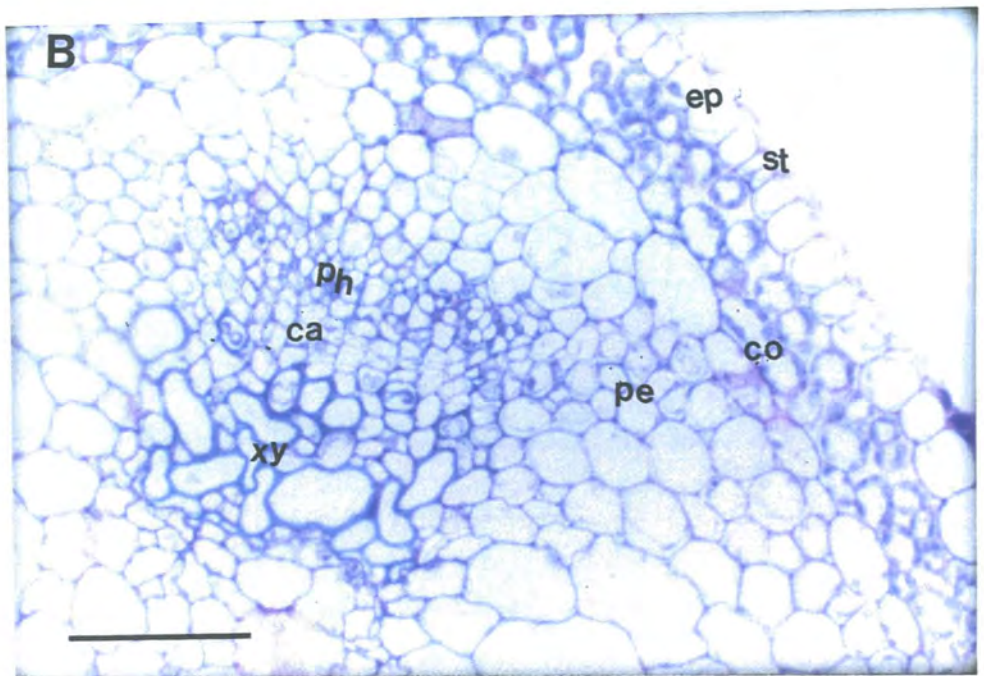
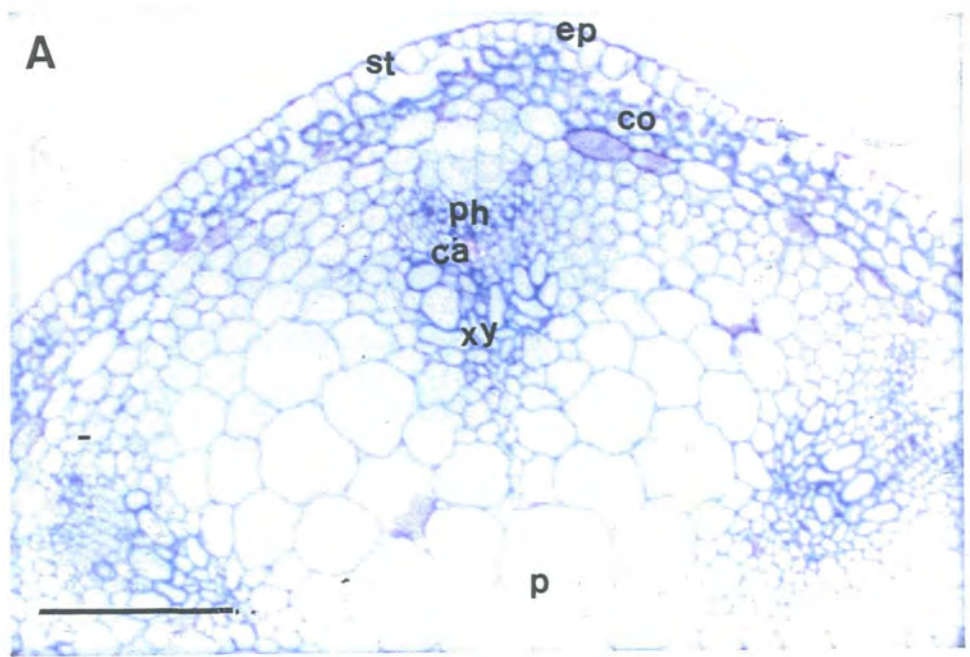


Plate.6.1 (A). Light micrograph of transverse section through stem of *Arabidopsis thaliana* (WT) seedlings, raised in absence of NaCl-stress conditions (control). ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μ m, B = 50 μ m).

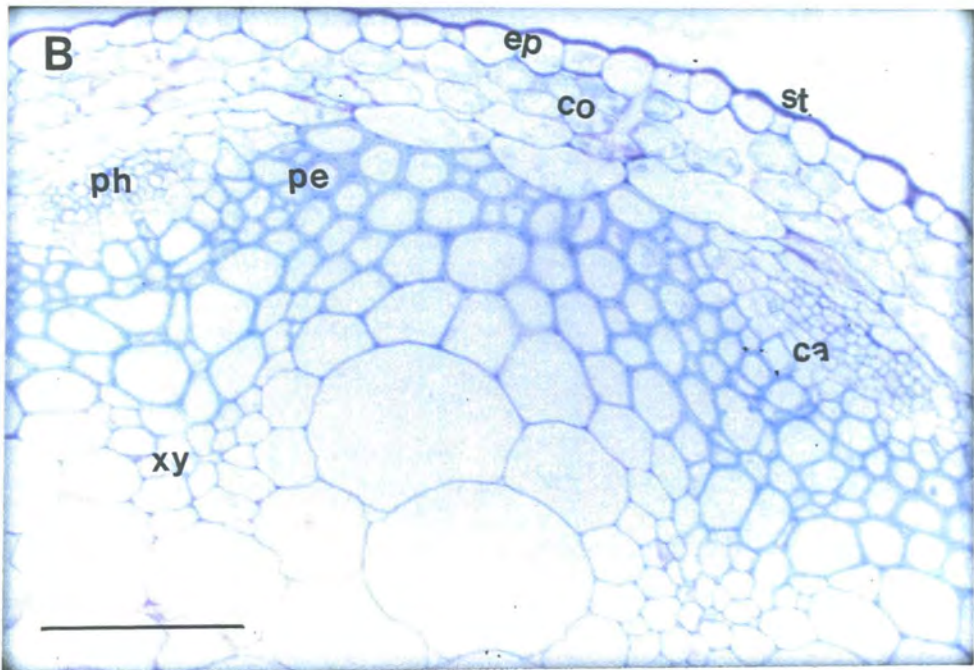
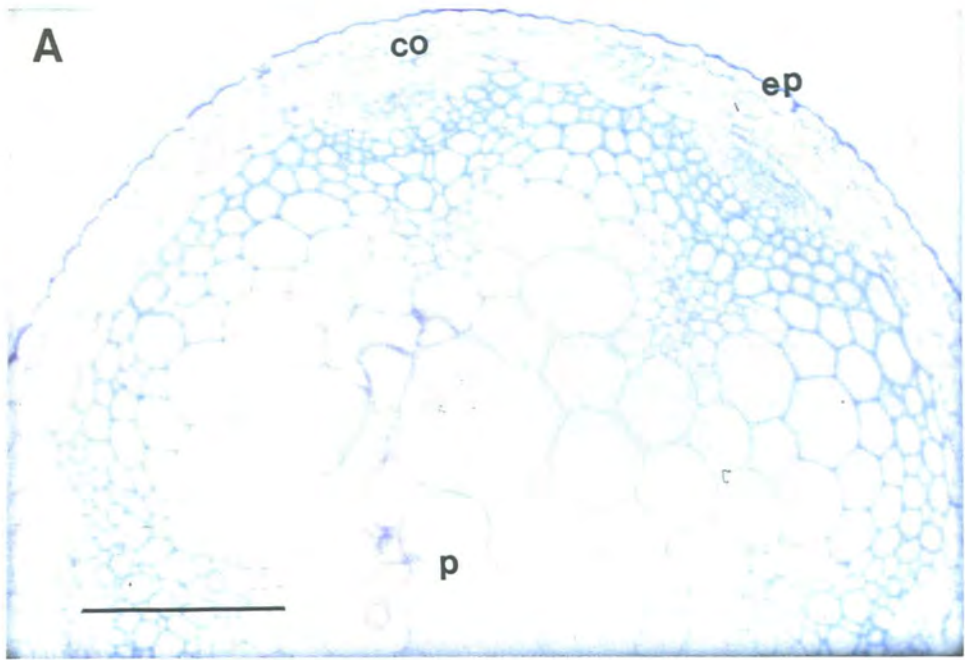


Plate 6.2 (A). Light micrograph of transverse section through stem of *Arabidopsis thaliana* (WT) seedlings, raised under NaCl-stress conditions (250 ppm).

Plate 6.2 (B). Note elongated, thinner cortical cells, lignified pericycle cells & larger xylem and pith cells than the control.

ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μ m, B = 50 μ m).

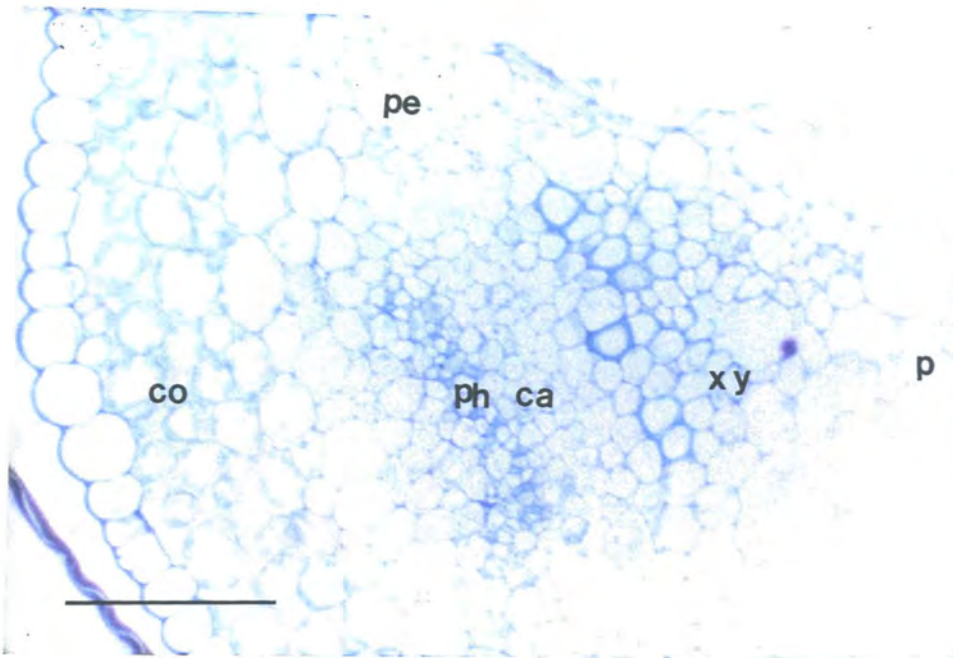
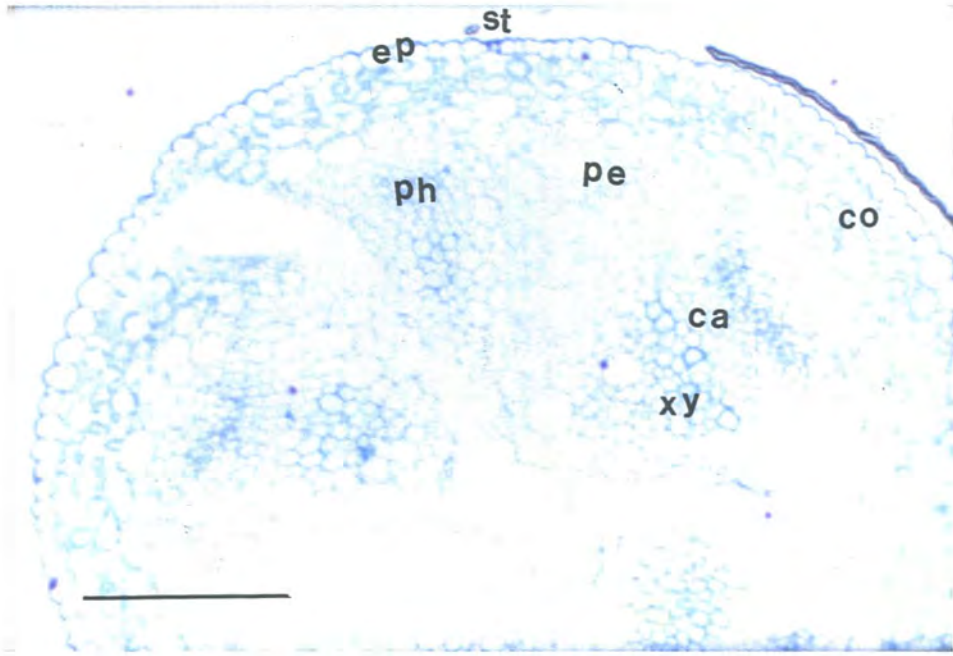


Plate.6.3 (A). Light micrograph of transverse section through stem of *Arabidopsis thaliana* (WT), seedlings, raised under NaCl-stress conditions (500 ppm).

Plate 6.3 (B). Note more cortex cell layers with larger cells, small, less lignified xylem cells & small pith cells than control. ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μ m, B = 50 μ m).

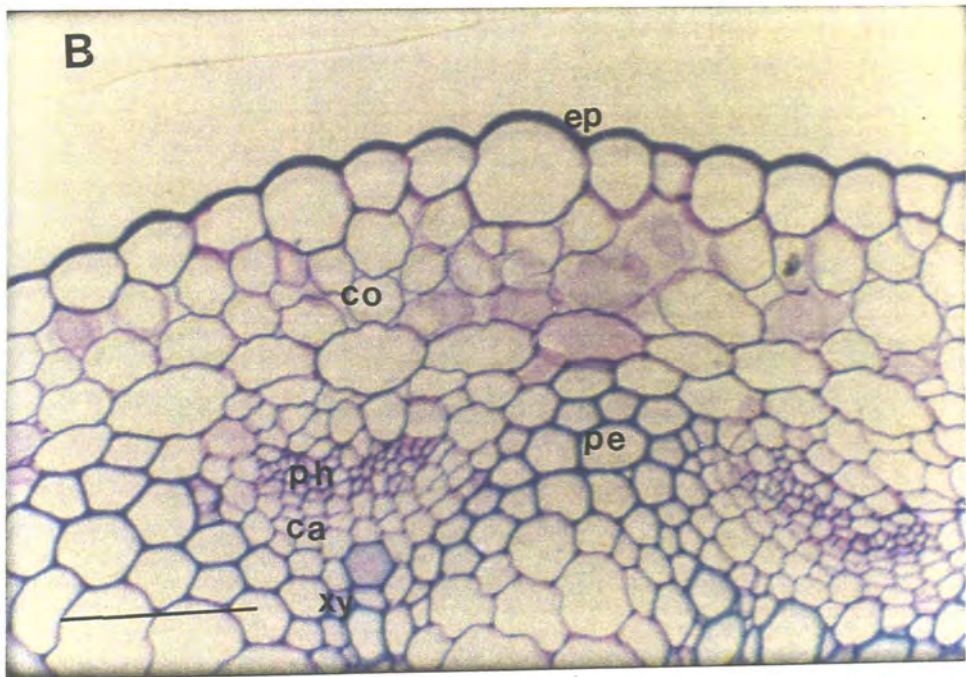
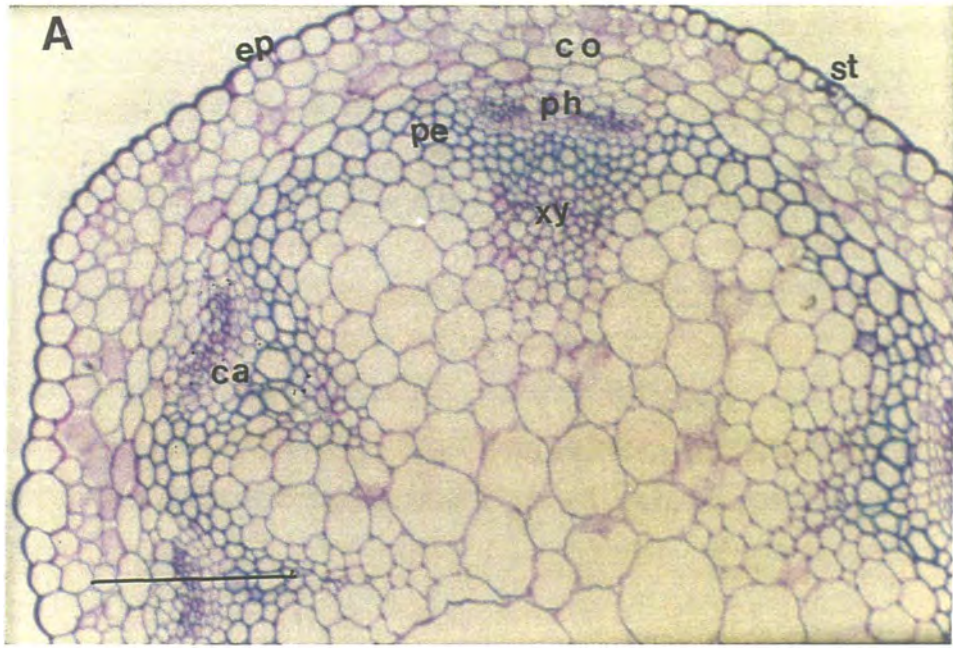


Plate 6.4 (A). Light micrograph of transverse section through stem of *Arabidopsis thaliana* (WT), seedlings, raised under NaCl-stress condition (1000 ppm).

Plate 6.4 (B). Note larger epidermal cells, more cortex layers with larger cells & more lignified pericycle and xylem cells than the control.

ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μ m, B = 50 μ m).

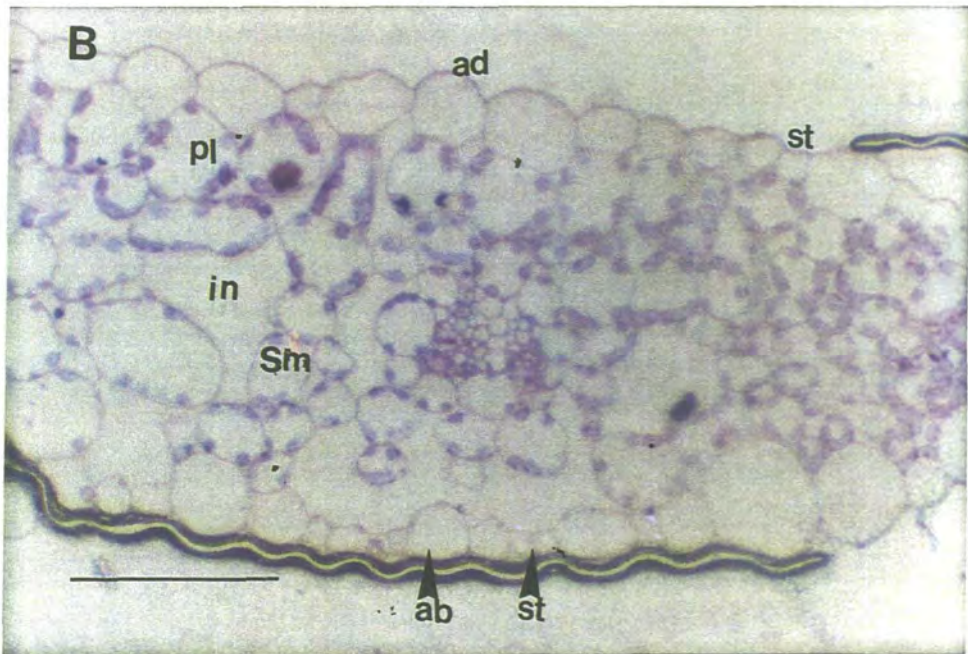
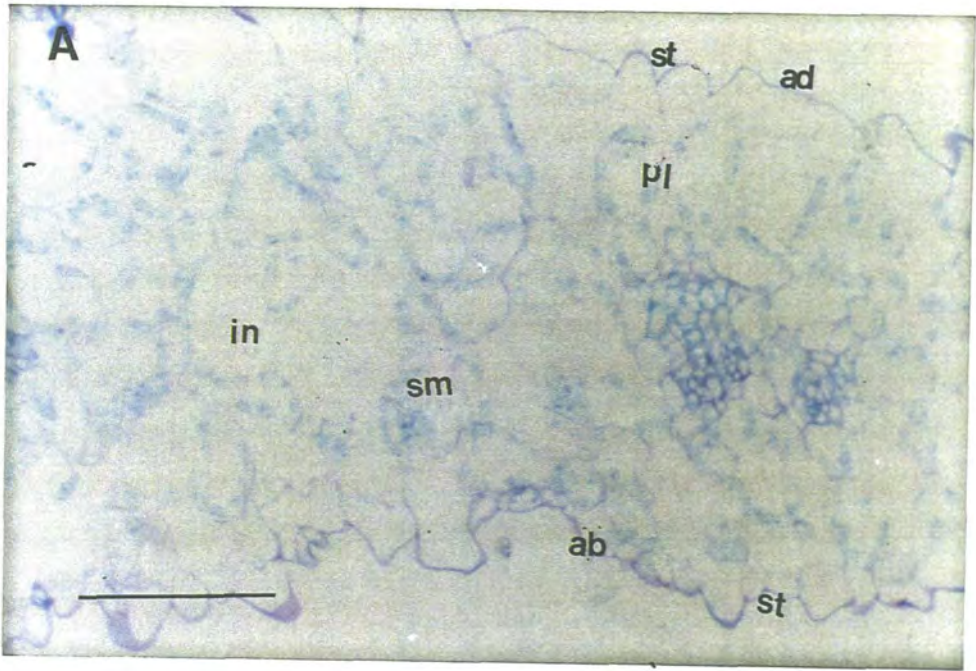


Plate 6.5 A- B. Light micrographs of transverse sections through leaf of *Arabidopsis thaliana* (WT) seedlings raised, under NaCl-stress conditions **A**: control; **B**: 250 ppm.

Plate 6.5 (B). Notice small spongy mesophyll cells with smaller intercellular spaces and more stomata on adaxial and abaxial surface than control.

ad, adaxial epidermis; ab, abaxial epidermis; pl palisade parenchyma; sp, spongy mesophyll; in, intercellular spaces; st, stomata. (Scale bars = 100 μ m).

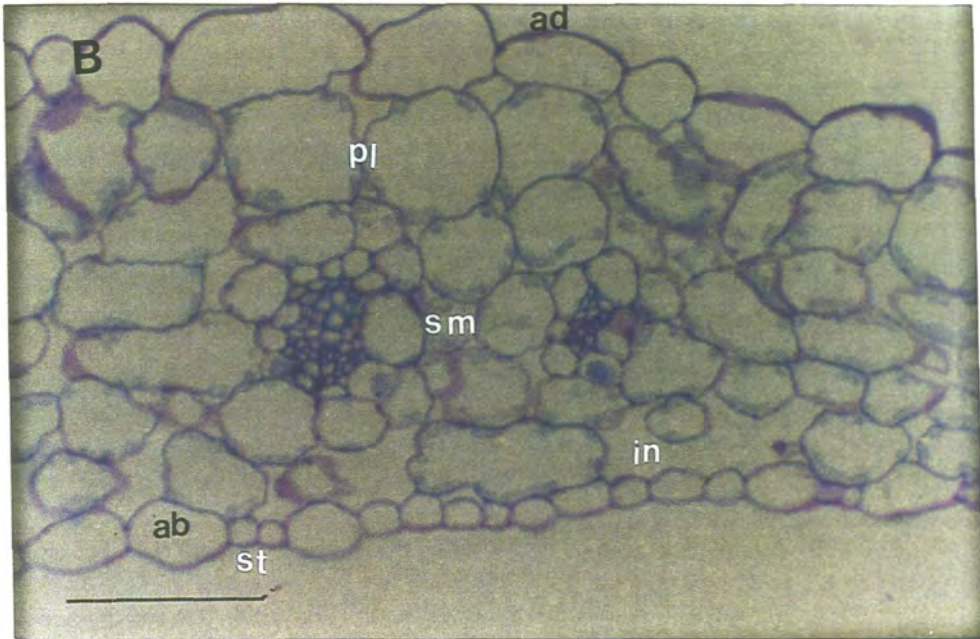
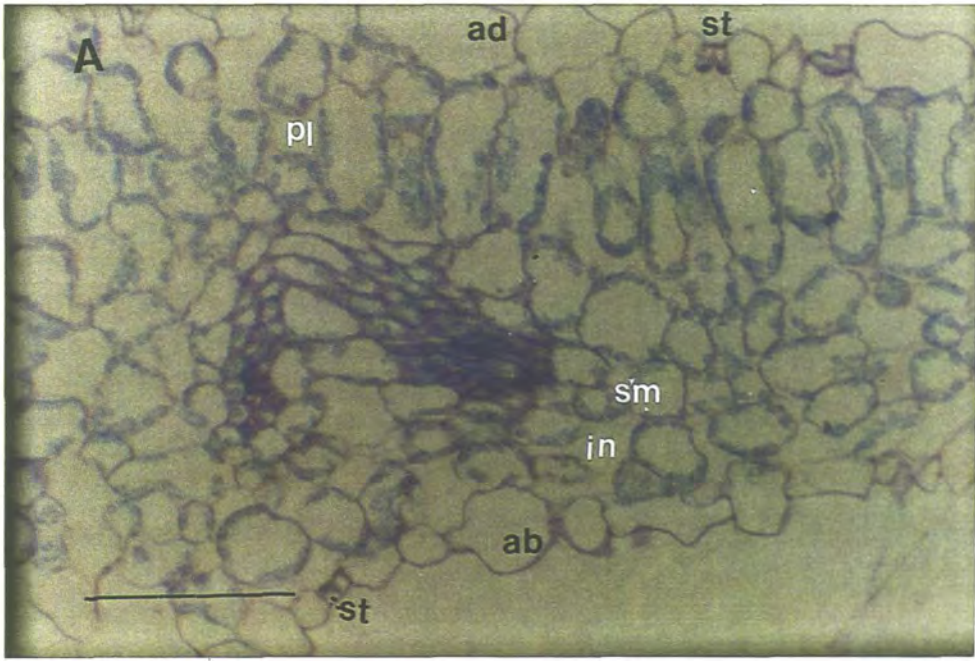


Plate 6.6 A- B. Light micrographs of transverse sections through leaf of *Arabidopsis thaliana* (WT) seedlings raised under NaCl-stress conditions A: 500 ppm; B: 1000 ppm.

Plate 6.6 (A). Notice more elongated palisade parenchyma with smaller intercellular spaces and compressed spongy mesophyll with smaller air spaces.

Plate 6.6 (B). Notice larger adaxial and smaller abaxial epidermal cells, roundish, larger palisade parenchyma with smaller intercellular spaces and compressed spongy mesophyll with smaller intercellular spaces.

ad, adaxial epidermis; ab, abaxial epidermis; pl, palisade parenchyma; sp, spongy mesophyll; in, intercellular spaces; st, stomata. (Scales bar = 100 μ m).

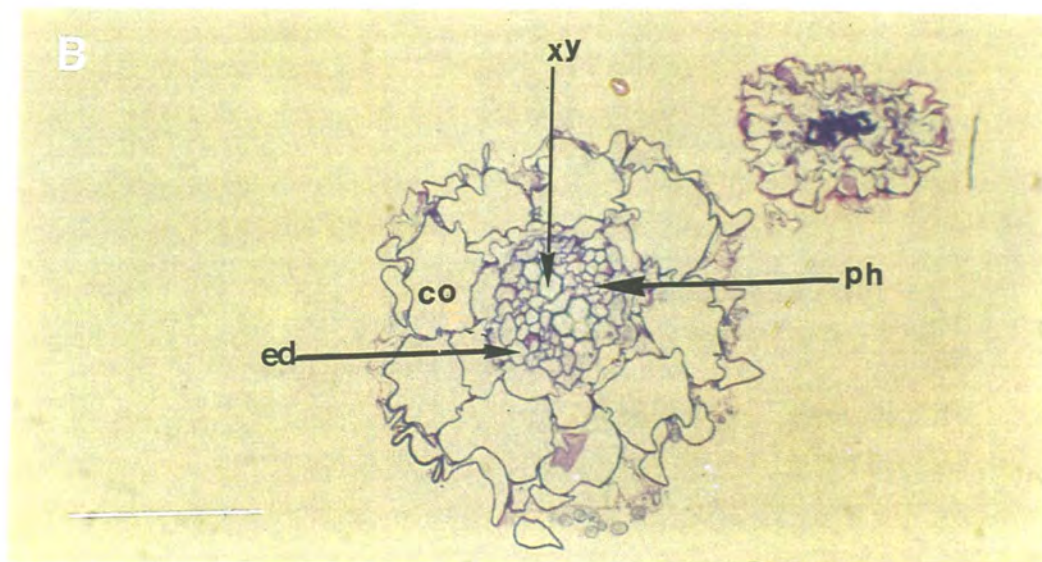
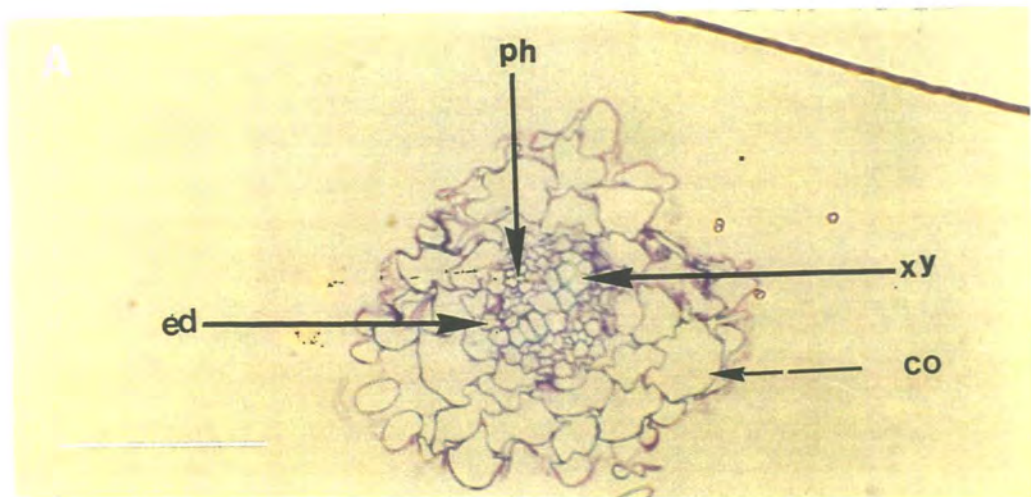


Plate 6.7 A- B. Light micrographs of transverse sections through root of *Arabidopsis thaliana* (WT) seedlings, raised under NaCl-stress conditions **A**: control; **B**:250 ppm.

Plate 6.7 (B). Notice larger cortical cells, lignified and undifferentiated pericycle and phloem. co, cortex; ed, endodermis; ph, phloem; xy, xylem. (Scale bars = 50 μ m).

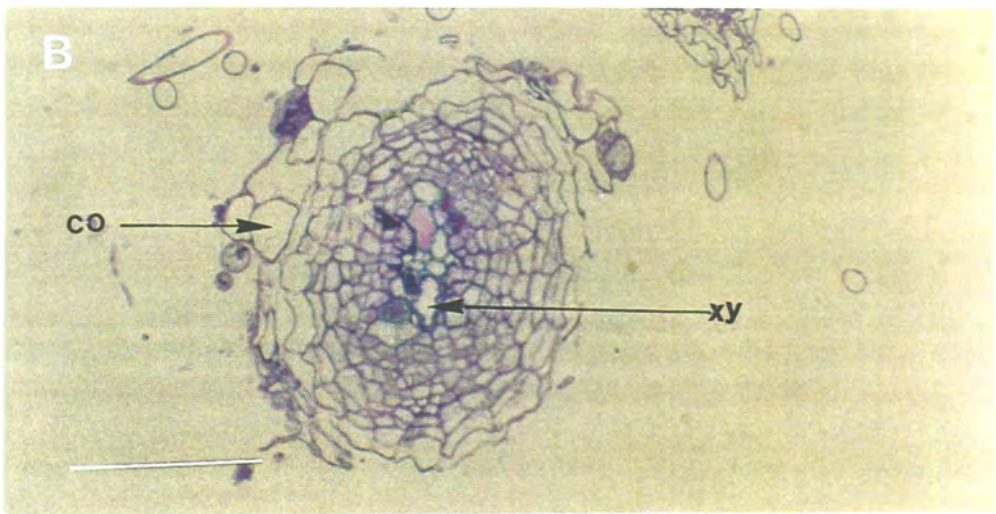
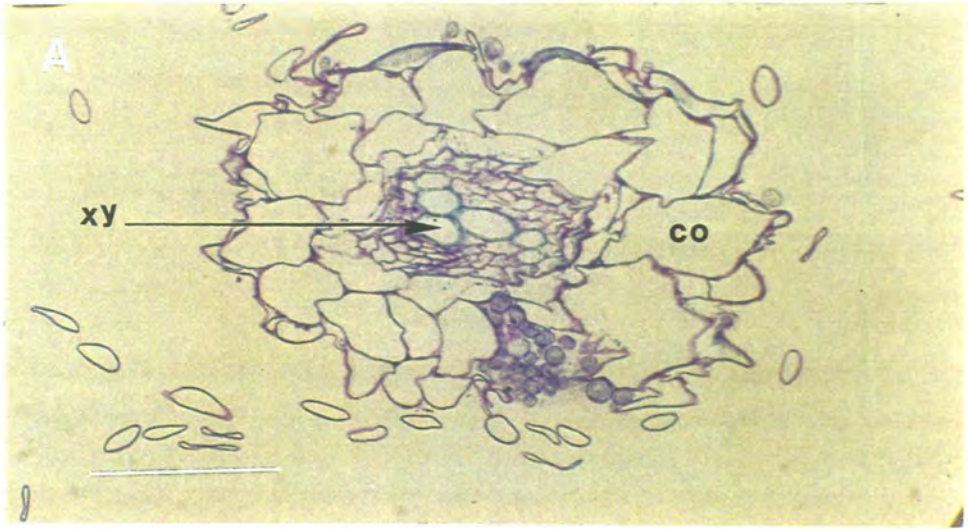


Plate 6.8 A- B. Light micrographs of transverse sections through root of *Arabidopsis thaliana* (WT) seedlings raised under NaCl-stress conditions A: 500 ppm; B: 1000 ppm.

Plate 6.8 (A). Notice larger cortical cells & more lignified pericycle and phloem.
 Plate 6.8 (B). Note disrupted cortical layers & larger stelar area than the control.
 co, cortex; xy, xylem. (Scale bar = 50 μ m).

Chapter VII

Effects of heavy metals on the sporophyte and gametophyte of

Arabidopsis thaliana

Experiment-1

7.1 Fruit-setting, seed-setting and seed-filling in *Arabidopsis thaliana* in response to high

This experiment was conducted to quantify the effects of heavy metals on fruit-setting, seed-setting and seed-filling. The details of results for every heavy metal are interpreted as below:

7.2 Effects of copper

Highly significant results for reduction in seeds set per pod ($f = 8.19$, $df = 5$, $p \leq 0.00$) and reduction in filled seeds per pod ($f = 11.89$, $df = 5$, $p \leq 0.00$) were observed. The data for fruit-setting could not be statistically analysed because of a few number of crosses. Comparing successful number of crosses with MS-SF x wild-SF which designated as a control, arithmetically the decline of 16.30, 22.22 and 27.00 % in pod set was respectively (Table-7.1, Fig. 7.1) recorded for crosses MS-HS x wild-HS, MS-SF x wild-HS and MS-HS x wild-SF.

Considering wild-SF as the control, the decline of 4.66 (MS-SF x wild-SF) to 56.78 % (MS-HS x wild-SF) and 1.60 (MS-HS x wild-HS) to 24.24 % (wild-HS) was noted for seed-setting and seed-filling respectively. Exceptionally the increase of 6.5 % against control (wild-SF) was observed in combination MS-SF x wild-SF in respect with seed-filling (Table-7.1, Fig. 7.2).

The data for fresh and dry plant weight could not be statistically analysed because of the low number of plants. Arithmetically loss (Table-7.2, Fig. 7.3) of 39.23 (Wild-HS) to 46.65 % (MS-HS) and 35.08 (wild-HS) to 46.56 % (MS-HS) was observed in plants under high copper stress conditions against their respective controls (MS-SF, wild-SF) for fresh and dry plant weights respectively.

7.2.1 Effects of aluminium

While observing the effects of aluminium for the fruit-setting, seed-setting and seed-filling (Table-7.3, Fig. 7.4) in *Arabidopsis*, the loss of 6.04% (MS-HS x wild-HS) to 22.03% (MS-SF x wild-HS) was noted for the fruit-setting among the crosses against control (MS-SF x wild-SF). The loss of 34.79% (wild-HS) to 49.26% (MS-HS x wild-SF) and 4.04% (MS-SF x wild-SF) to 21.54% (MS-HS x wild-SF) was quantified respectively for seed-setting and seed-filling (Fig. 7.5) comparatively to the control (wild-SF). The increase of 2.95 % was recorded for the combination MS-SF x wild-SF against the control (wild-SF). Assessing the plant losses (Table-7.4, Fig. 7.6) for aluminium, the loss of 40.53% (MS-HS) to 42.36% (wild-HS) and 32.67% (MS-HS) to 41.39% (wild-HS) was recorded against controls (MS-SF, wild-SF) in respect of plant fresh and dry weight respectively.

The data for fruit-setting and plant losses could not be statistically analysed because of specific number of crosses and low number of plants respectively. However highly significant results were noted for reduction of seed-setting ($f = 6.55$, $df = 5$, $p \leq 0.001$) and seed-filling ($f = 7.18$, $df = 5$, $p \leq 0.00$).

7.2.2 Effect of zinc sulphate

As described in the materials and methods, a high level of $ZnSO_4$ (300 ppm)

was chosen to quantify its impact on the fruit-setting, seed-setting and seed-filling in *Arabidopsis thaliana*. The results (Table-7.5, Fig. 7.7) indicate that the loss of 12.73% (MS-HS x wild-HS) to 20.91% (MS-SF x wild-HS) was observed in regard with fruit-setting. Highly significant results were recorded for seed-setting ($f = 8.12$, $df = 5$, $p \leq 0.001$), whereas nonsignificant results were recorded for seed-filling ($f = 1.72$, $df = 5$, $p \leq 0.169$). The loss of 2.28% (MS-SF x wild-SF) to 48.40 % (MS-HS x wild-SF) and 12.17% (wild-HS) to 18.21% (MS-SF x wild-HS) were noted respectively for seed-setting and seed-filling (Fig. 7.8) relatively to the control (wild-SF). In respect of plant fresh and dry weight, (Table-7.6, fig. 7.9) a calculated decline of 48.27% (wild-HS) to 57.91% (MS-HS) and 53.90% (wild-HS) to 54.89% (MS-HS) was noted respectively for both traits against their respective controls (MS-SF, wild-SF).

7.2.3 Effect of cadmium sulphate

In order to evaluate the effects of cadmium sulphate on the fruit-setting, seed-setting and seed-filling in *Arabidopsis thaliana*, a high concentration (200 ppm) was chosen to put the pollen donors and recipient plants under stress conditions as described earlier in materials and methods. After crossing under stress and stress-free regimes the fruit-setting, seed-setting and seed-filling were studied.

The loss of (Table-7.7, Fig. 7.10) 9.12% (MS-HS x CAD1-SF) to 29.20% (MS-SF x CAD1-HS) was quantified for fruit-setting. Statistically highly significant results for reductions in seed-setting ($f = 23.79$, $df = 5$, $p \leq 0.000$) and seed-filling ($f = 15.22$, $df = 5$, $p \leq 0.000$) were noted. The significant losses of 28.77% (MS-HS x CAD1-SF) to 48.11% (MS-SF x CAD1-HS) and 10.00% (MS-HS x CAD1-SF) to 20.60% (MS-HS x CAD1-HS) were recorded for seed-setting and seed-filling. The

increase of 12.26% and 1.82% was noted against control (CAD1-SF) respectively for seed-setting and seed-filling. Losses of 37.60% (CAD1-HS) to 38.44% (MS-HS) and 42.21% (MS-HS) to 54.28% (CAD1-HS) were recorded for fresh and dry plant weight (Table-7.8, Fig. 7.12).

Table-7.1: Effects of copper sulphate high stress (20 ppm) on the fruit-setting, seed-setting and seed-filling in *Arabidopsis*. SD in parenthesis.

Combination	Crosses attempted	Fruit setting (%)	% inc. (+) or dec. (-)	Seeds set per pod	% inc. (+) or dec. (-)	Filled seed (%)	% inc. (+) or dec. (-)
MS-HS x wild-HS	49	81.63	-16.30	33.00 (8.75)	-30.80	77.93 (7.22)	-1.60
MS-HS x wild-SF	31	70.97	-27.00	20.40 (5.68)	-56.78	74.22 (6.66)	-6.29
MS-SF x wild-HS	32	75.00	-22.22	22.80 (4.21)	-51.69	75.75 (5.79)	-4.36
MS-SF x wild-SF	72	97.22	-	45.00 (5.79)	-4.66	84.40 (9.52)	+6.56
Wild-HS	-	-	-	27.20 (7.46)	-42.37	77.28 (5.26)	-24.24
Wild-SF	-	-	-	47.20 (10.38)	-	79.20 (3.32)	-
F (df)	-	-	-	8.19 (5)	-	11.89 (5)	-
P ≤	-	-	-	0.00	-	0.00	-

Table-7.2: Effects of copper sulphate high stress (20 ppm) on the plant fresh and dry weight in *Arabidopsis thaliana*.

Plant group	Fresh plant weight (g)	% inc. (+) or dec. (-)	Dry plant weight (g)	% inc. (+) or dec. (+)
MS-HS	1.2350	-46.65	0.5467	-46.56
MS-SF	2.3150	-	1.0136	-
Wild-HS	0.9535	-39.23	0.5312	-35.08
Wild-SF	1.5691	-	0.8182	-

Table-7.3: Effects of aluminium sulphate high stress (500 ppm) on the fruit-setting, seed-setting and seed-filling in *Arabidopsis*. SD in parenthesis.

Combination	Crosses attempted	Fruit setting (%)	% inc. (+) or dec. (-)	Seeds per pod	% inc. (+) or dec. (-)	Filled seed (%)	% inc. (+) or dec. (-)
MS-HS x wild-HS	59	86.44	-6.04	26.00 (5.70)	-35.96	79.88 (3.62)	-15.20
MS-HS x wild-SF	42	83.00	-9.78	20.60 (6.31)	-49.26	73.91 (5.60)	-21.54
MS-SF x wild-HS	35	71.43	-22.03	25.40 (6.46)	-37.44	74.70 (7.87)	-20.70
MS-SF x wild-SF	70	92.00	-	41.80 (5.02)	+2.95	90.39 (7.25)	-4.04
Wild-HS	-	-	-	26.40 (10.66)	-34.79	80.95 (3.25)	-14.06
Wild-SF	-	-	-	40.60 (10.21)	-	94.20 (2.52)	-
F (df)	-	-	-	6.55 (5)	-	7.18 (5)	
P ≤	-	-	-	0.001	-	0.000	

Table-7.4: Effects of aluminium sulphate high stress (500 ppm) on plant fresh and dry weight of *Arabidopsis*.

Plant group	Fresh plant weight (g)	% inc. (+) or dec. (-)	Dry plant weight (g)	% inc. (+) or dec. (-)
MS-HS	0.9698	-40.53	0.5213	-32.67
MS-SF	1.6310	-	0.7769	-
Wild-HS	1.6213	-42.36	0.7926	-41.39
Wild-SF	2.8130	-	1.3523	-

Table-7.5: Effects of zinc sulphate high stress (300 ppm) on fruit-setting, seed-setting and seed-filling in *Arabidopsis*. SD in parenthesis.

Combination	Crosses attempted	Fruit setting (%)	% inc. (+) or dec. (-)	Seeds set pod	% inc. (+) or dec. (-)	Filled seed (%)	% inc. (+) or dec. (-)
MS-HS x wild-HS	47	85.11	-12.73	34.00 (6.59)	-22.37	79.49 (2.70)	-13.18
MS-HS x wild-SF	51	80.39	-17.57	22.60 (3.97)	-48.40	76.90 (4.35)	-16.01
MS-SF x wild-HS	35	77.14	-20.91	26.20 (3.03)	-40.18	74.89 (2.51)	-18.21
MS-SF x wild-SF	81	97.53	-	42.80 (3.56)	-2.28	92.12 (2.32)	+0.61
Wild-HS	-	-	-	30.20 (10.75)	-31.05	80.42 (3.81)	-12.17
Wild-SF	-	-	-	43.80 (8.98)	-	91.56 (6.89)	-
F (df)	-	-	-	8.12 (5)	-	1.72 (5)	
P ≤	-	-	-	0.001	-	0.169	

Table-7.6: Effects of zinc sulphate high stress (300 ppm) on fresh and dry plant weight in *Arabidopsis*

Plant group	Fresh plant weight (g)	% inc. (+) or dec. (-)	Dry plant weight (g)	% inc. (+) or dec. (-)
MS-HS	1.0621	-57.91	0.5391	-54.89
MS-SF	2.5235	-	1.1924	-
Wild-HS	0.5132	-48.27	0.2449	-53.90
Wild-SF	0.9921	-	0.5323	-

Table-7.7: Effects of cadmium sulphate high stress (200 ppm) on the fruit-setting, seed-setting and seed-filling in *Arabidopsis*. SD in parenthesis.

Combination	Crosses attempted	Fruit setting (%)	% inc. (+) or dec. (-)	Seeds set per pod	% inc. or dec. (-)	Filled seed (%)	% inc. (+) or dec. (-)
MS-HS x CAD1-HS	49	71.42	-23.96	27.20 (3.19)	-35.84	71.63 (5.17)	-20.60
MS-HS x CAD1-SF	41	85.36	-9.12	30.20 (6.14)	-28.77	81.18 (8.15)	-10.00
MS-SF x CAD1-HS	39	66.67	-29.20	22.00 (1.87)	-48.11	66.35 (6.18)	-26.44
MS-SF x CAD1-SF	45	93.93	-	67.60 (5.68)	+12.26	91.86 (4.35)	+1.82
CAD1-HS	-	-	-	26.20 (2.86)	-38.20	74.90 (7.20)	-16.97
CAD1-SF	-	-	-	42.40 (5.02)	-	90.21 (2.27)	-
F (df)	-	-	-	23.79 (5)	-	15.22 (5)	
P ≤	-	-	-	0.000	-	0.000	

Table-7.8: Effects of cadmium sulphate high stress (200 ppm) on plant fresh and dry weight in *Arabidopsis*

Plant group	Fresh plant weight (g)	% inc. (+) or dec. (-)	Dry plant weight (g)	% inc. (+) or dec. (-)
MS-HS	0.9539	-38.44	0.5421	-42.21
MS-SF	1.5496	-	0.9381	-
CAD1-HS	1.3112	-37.60	0.5012	-54.28
CAD1-SF	2.1014	-	1.0963	

Experiment-2

7.3 Effect of heavy metals on pollen germination & pollen tube growth

Four metallic salts viz. aluminium sulphate, zinc sulphate, iron sulphate and copper sulphate, were chosen to study their inhibitory effect on the pollen germination and pollen tube growth. Highly significant differences were noted among metallic levels and the control (no added metal).

7.3.1 Effect of aluminium sulphate

Five levels (10, 20, 30, 40, 50 ppm) of aluminium sulphate added in the pollen germinating medium, gave highly significant differences in respect of pollen germination ($f = 46.69$, $df = 5$, $p \leq 0.001$) and pollen tube length ($f = 72.68$, $df = 5$, $p \leq 0.001$). The inhibitory effect (Table-7.9) was in a straight line slope (Figs. 7.13 , 7.16), and varied from 17.46% (10 ppm) to 63.68% (50 ppm) for pollen germination and from 44.66% (0 ppm) to 69.69% (50 ppm) for tube length. Inhibitory percentages were calculated against the performance of the control i.e. standard medium (SM).

7.3.2 Effect of zinc sulphate

For the effect of zinc sulphate highly significant differences for pollen germination ($f = 264.78$, $df = 5$, $p \leq 0.001$) and pollen tube length ($f = 52.39$, $df = 5$, $p \leq 0.001$) were recorded.

The extent of inhibition (Table-7.10, Fig. 7.13, 7.16) ranged from 29.30% (10 ppm) to 98.93% (50 ppm) and from 40.98% (10 ppm) to 92.57 (50 ppm) respectively for pollen germination and pollen tube length.

7.3.3 Effect of ferrous sulphate

Highly significant differences were noted statistically for pollen germination ($f = 79.86$, $df = 5$, $p \geq 0.001$) and pollen tube length ($f = 127.03$, $df = 5$, $p \leq 0.001$) for different levels of ferrous sulphate added to pollen germinating medium. The inhibitory effect (Table- 7.11, Fig. 7.13, 7.16) ranged from 32.78% to (10 ppm) to 93.12% (50 ppm) and from 25.44% (10 ppm) to 66.14% (50 ppm) respectively for pollen germination and tube length.

7.3.4 Effect of copper sulphate

As already mentioned, copper was highly inhibitory at low concentrations to pollen germination, and so it was included from 1 to 5 ppm to study its degree of inhibition.

Highly significant (Table-7.12) effects were recorded for PG ($f = 26.93$, $df = 5$, $p \leq 0.001$) and PTL ($f = 193.98$, $df = 5$, $p \leq 0.001$) for its inclusion in germinating medium. The inhibitory impacts (Table-7.12, Fig. 7.14, 7.15) varied from 32.06% (1 ppm) to 54.40 % (5 ppm) and 55.72% (1ppm) to 64.27% (5 ppm) for pollen germination and pollen tube length respectively.

Table-7.9: Effect of aluminium sulphate levels on pollen germination and growth in *Arabidopsis thaliana* (wild type).

SM plus Al-sulphate	Pollen germination (%)	Inhibition (%)	Pollen tube length (μm)	Inhibition (%)
10 ppm	55.14 \pm 5.05	17.46	391.21 \pm 13.56	44.46
20 ppm	37.87 \pm 6.23	43.31	339.73 \pm 28.48	52.03
30 ppm	24.85 \pm 2.61	62.80	331.19 \pm 24.95	53.23
40 ppm	24.52 \pm 3.92	63.29	241.39 \pm 24.95	65.91
50 ppm	24.26 \pm 5.38	63.68	214.67 \pm 9.14	69.69
SM (control)	66.81 \pm 3.20	-	708.26 \pm 84.59	-
F (df)	46.69 (5)	-	72.68 (5)	-
P \leq	0.001	-	0.001	-

Table-7.10: Effect of zinc sulphate levels on the pollen germination and growth in *Arabidopsis thaliana* (wild type).

SM plus Zn-sulphate	Pollen germination(%)	Inhibition (%)	Pollen tube length (μm)	Inhibition (%)
10 ppm	51.1	29.30	412.92	40.98
20 ppm	32.03	55.68	359.37	48.63
30 ppm	26.19	63.76	277.55	60.33
40 ppm	2.82	96.09	275.20	60.66
50 ppm	0.77	98.93	51.95	92.57
SM (control)	72.28	-	699.68	-
F (df)	264.78 (5)	-	52.39 (5)	-
P \leq	0.001	-	0.001	-

Table-7.11: Effect of ferrous sulphate levels on the pollen germination and tube growth in *Arabidopsis thaliana* (wild type).

SM plus Fe-sulphate	Pollen germination(%)	Inhibition (%)	Pollen tube length (μm)	Inhibition (%)
10 ppm	49.25 \pm 2.20	32.78	551.74 \pm 53.26	25.44
20 ppm	47.02 \pm 6.23	35.82	529.90 \pm 60.35	28.39
30 ppm	45.31 \pm 5.66	38.16	484.52 \pm 61.63	34.52
40 ppm	40.98 \pm 2.33	44.06	475.62 \pm 58.23	35.86
50 ppm	5.04 \pm 1.23	93.12	250.58 \pm 42.37	66.14
SM (control)	73.27 \pm 7.00	-	740.01 \pm 41.50	-
F (df)	79.86 (5)	-	127.03 (5)	-
P \leq	0.001	-	0.001	-

Table-7.12: Effect of copper sulphate levels on the pollen germination and tube growth in *Arabidopsis thaliana* (wild type).

SM plus Cu-sulphate	Pollen germination(%)	Inhibition (%)	Pollen tube length (μm)	Inhibition (%)
1 ppm	50.63 \pm 5.59	32.06	309.67 \pm 39.79	55.72
2 ppm	45.53 \pm 4.88	38.91	269.20 \pm 37.00	61.51
3 ppm	40.58 \pm 9.93	45.55	255.76 \pm 36.88	63.43
4 ppm	35.10 \pm 5.86	52.90	250.67 \pm 23.82	64.16
5 ppm	33.98 \pm 2.25	54.40	249.89 \pm 25.29	64.27
SM (control)	74.53 \pm 4.98	-	699.43 \pm 64.90	-
F (df)	26.93 (5)	-	193.98 (5)	-
P \leq	0.001	-	0.001	-

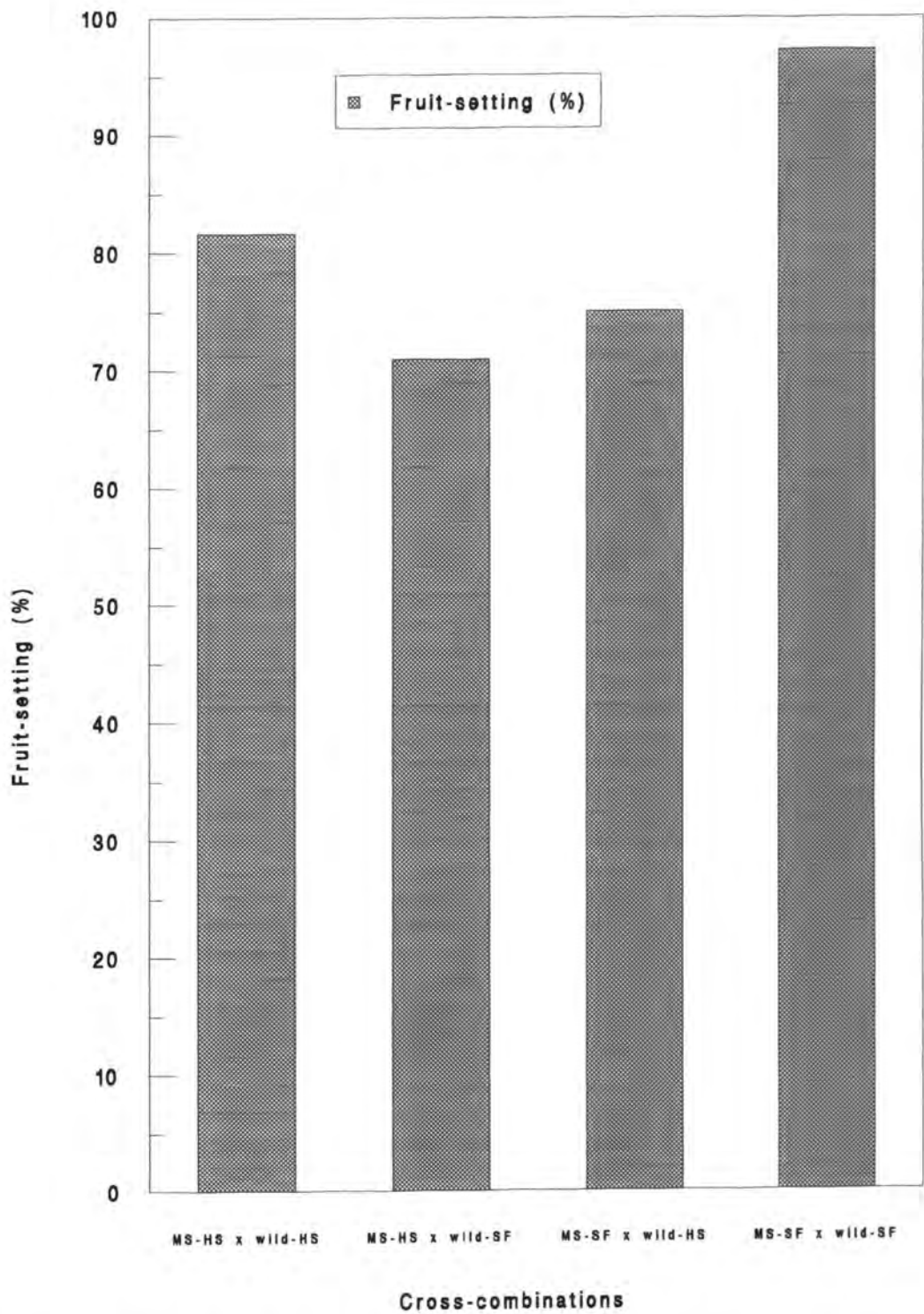


Fig. 7.1. Effect of copper sulphate high-stress (20 ppm). on the fruit-setting (%) of *Arabidopsis thaliana*.

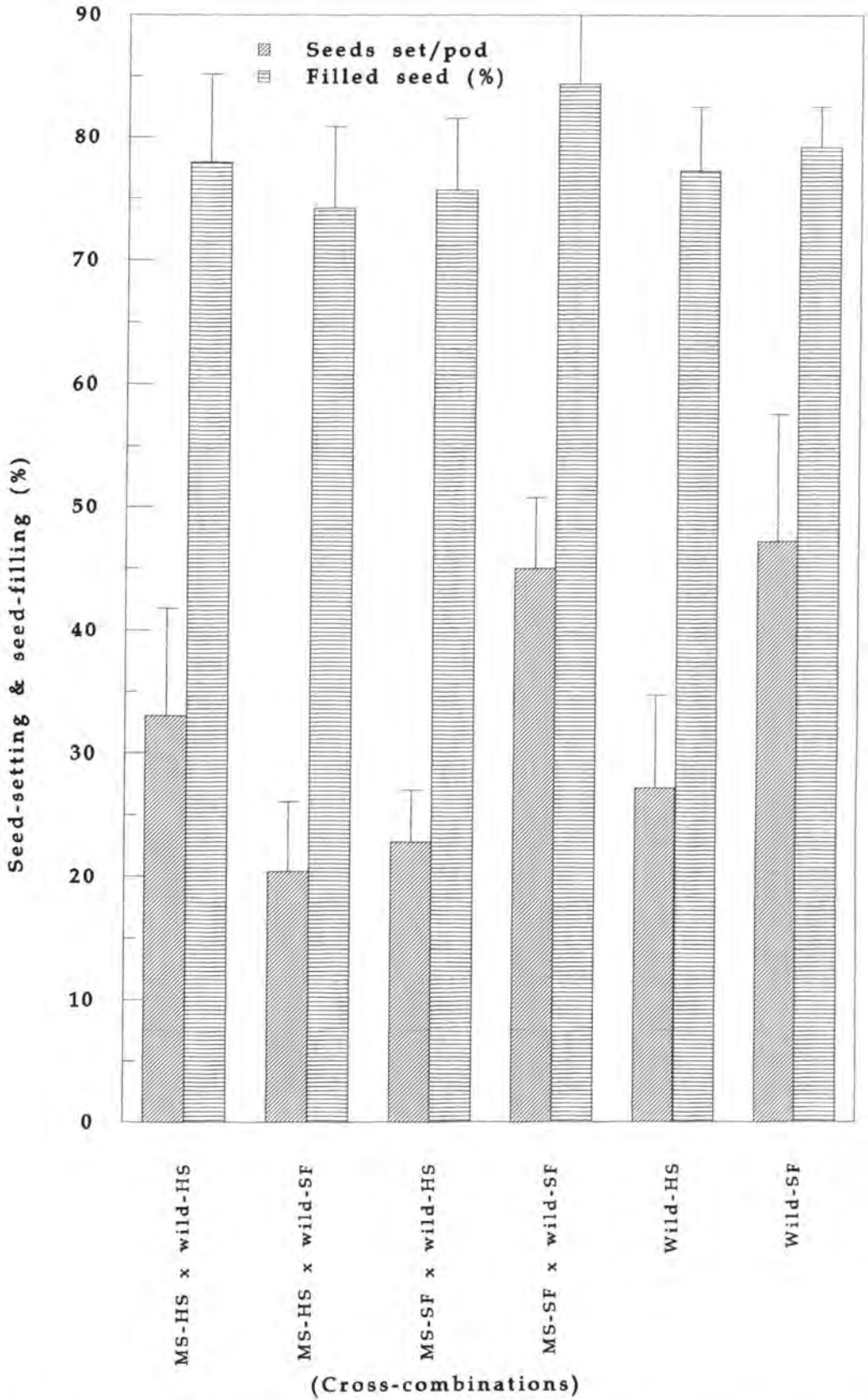


Fig. 7.2. Effect of copper sulphate high-stress (20 ppm) on the seed-setting and seed-filling in *Arabidopsis thaliana*.

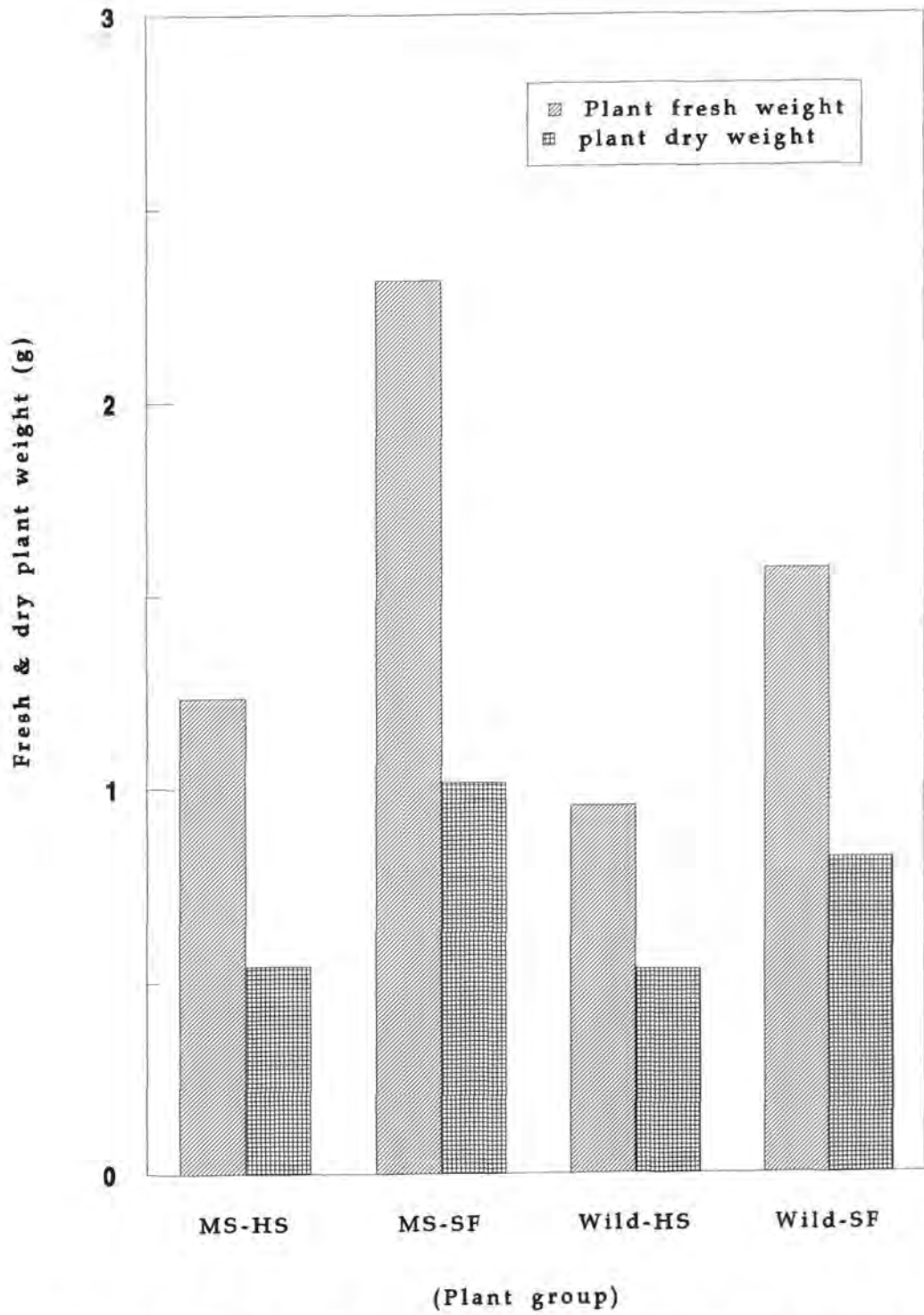
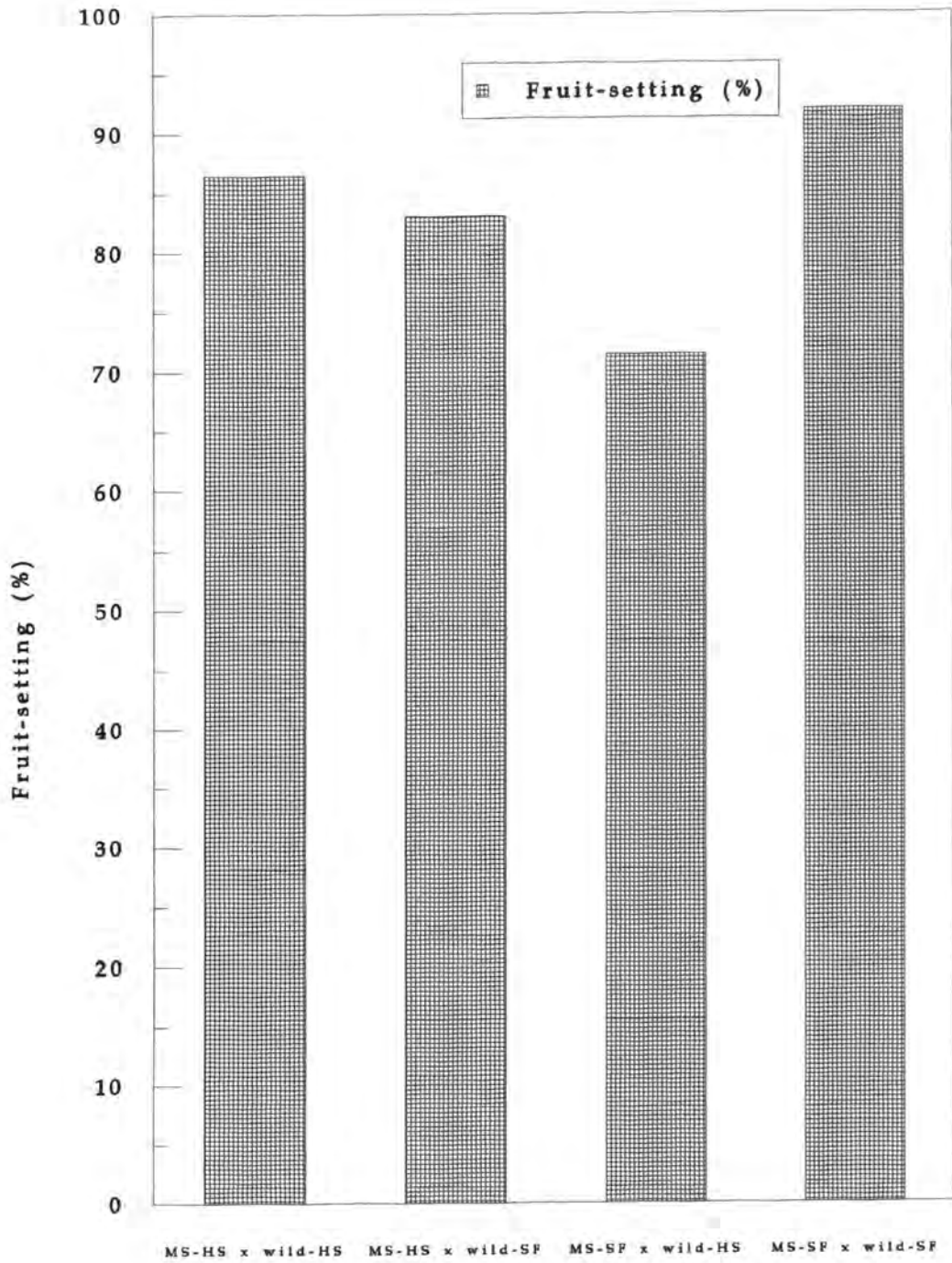


Fig.7.3. Effect of copper sulphate high-stress (20 ppm) on the plant fresh and dry weight of *A. thaliana*.



(Cross-combinations)

Fig.7.4. Effects of aluminium high-stress (500 ppm) on the fruit-setting (%) of *A. thaliana*.

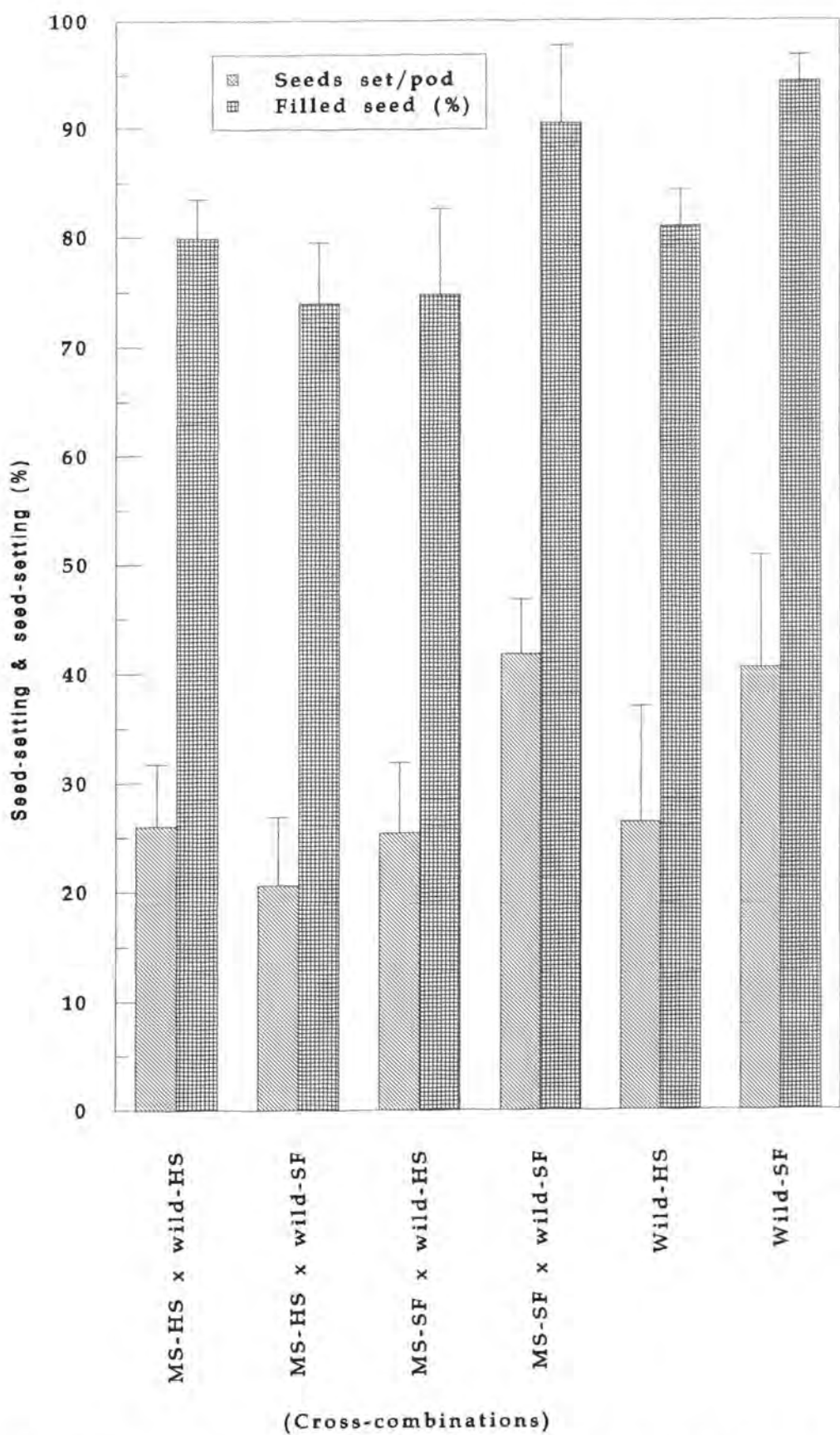


Fig. 7.5. Effect of aluminium sulphate high-stress (500 ppm) on the seed-setting and seed-filling of *Arabidopsis thaliana*.

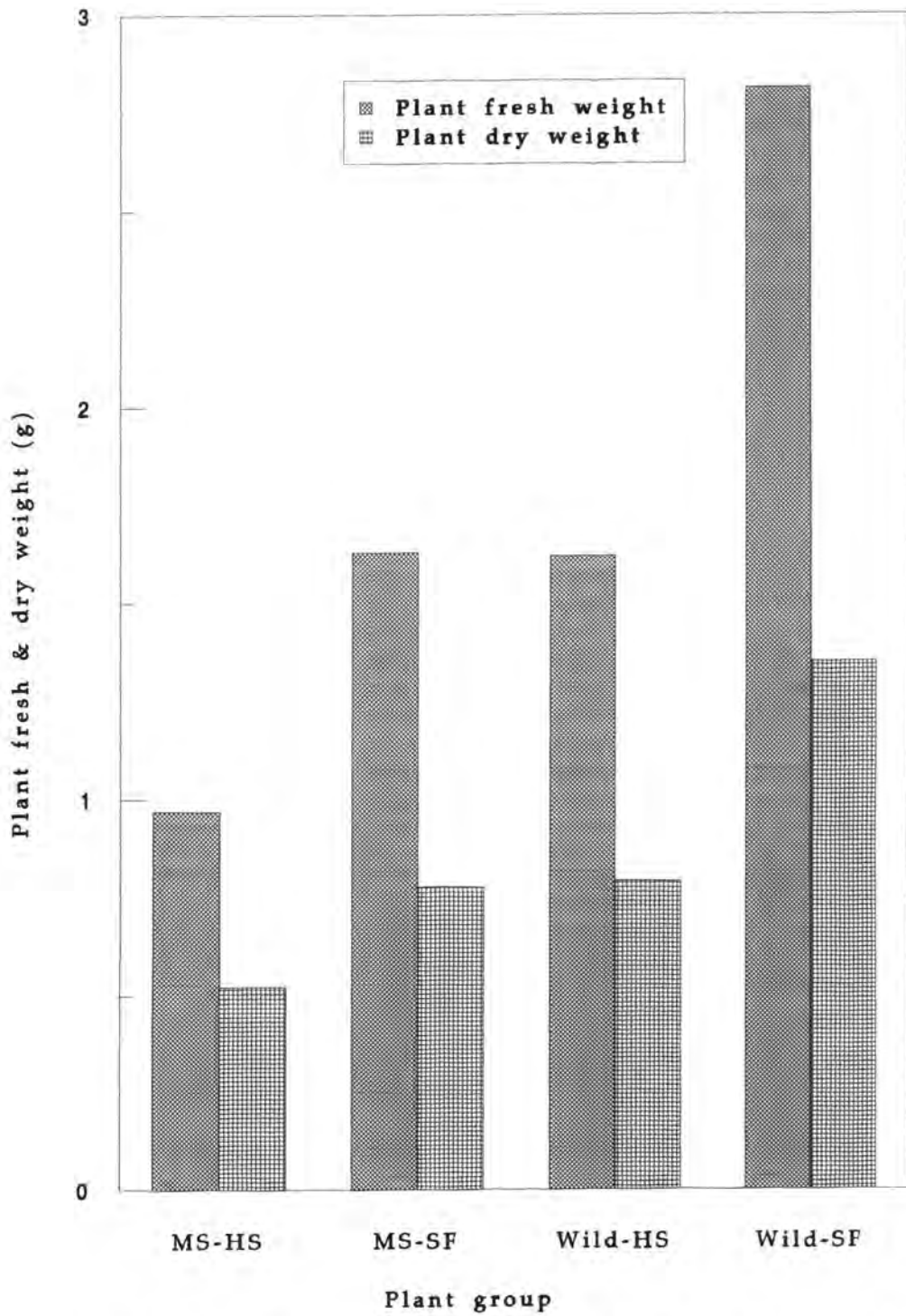


Fig. 7.6. Effect of aluminium sulphate high-stress (500 ppm) on the plant fresh and dry weight of *A. thaliana*.

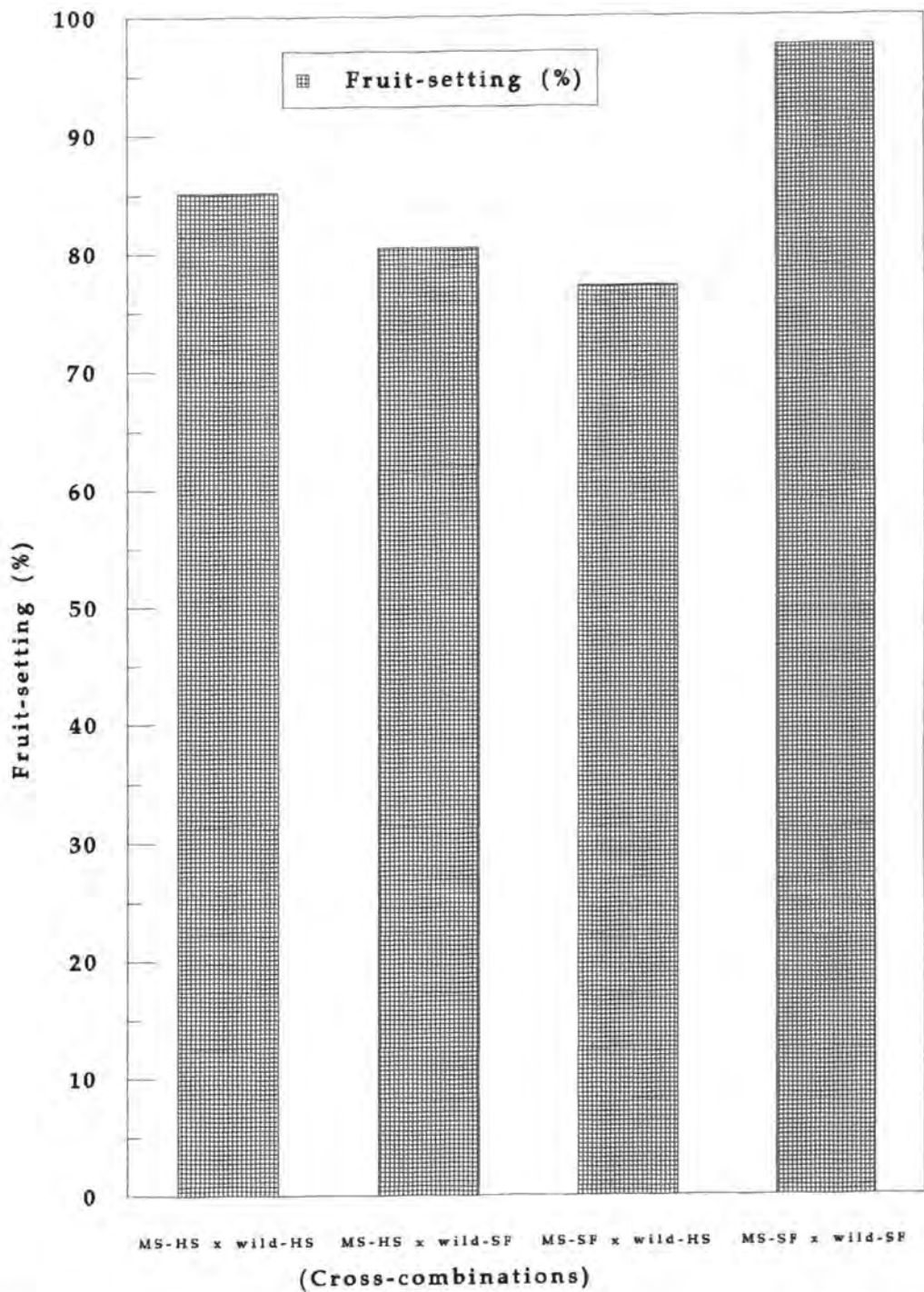


Fig. 7.7. Effect of zinc sulphate high-stress (300 ppm) on the fruit-setting of *A. thaliana*. (wild type)

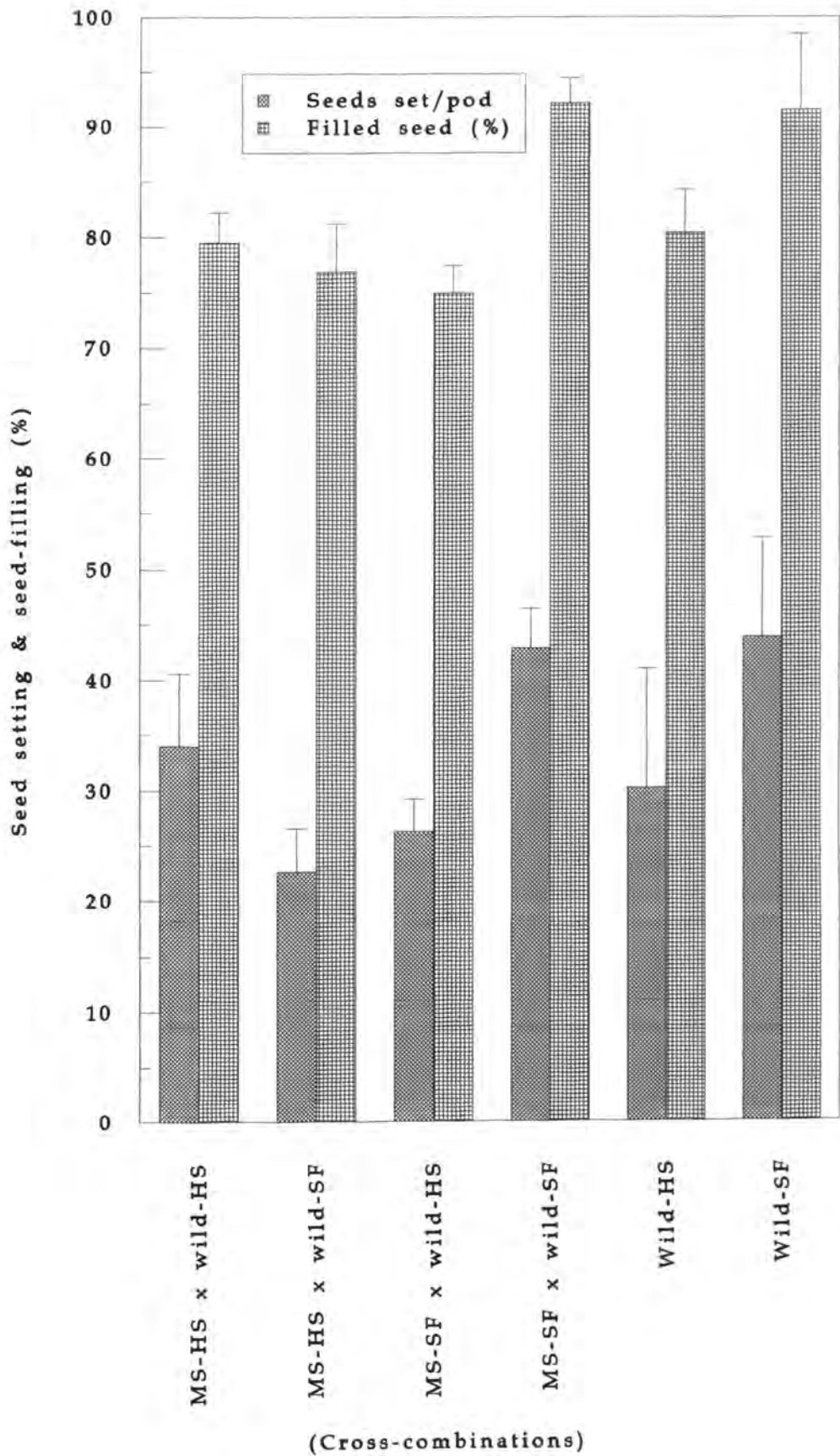


Fig. 7.8. Effect of zinc sulphate high stress (300 ppm) on the seed-setting and seed-filling of *A. thaliana*.

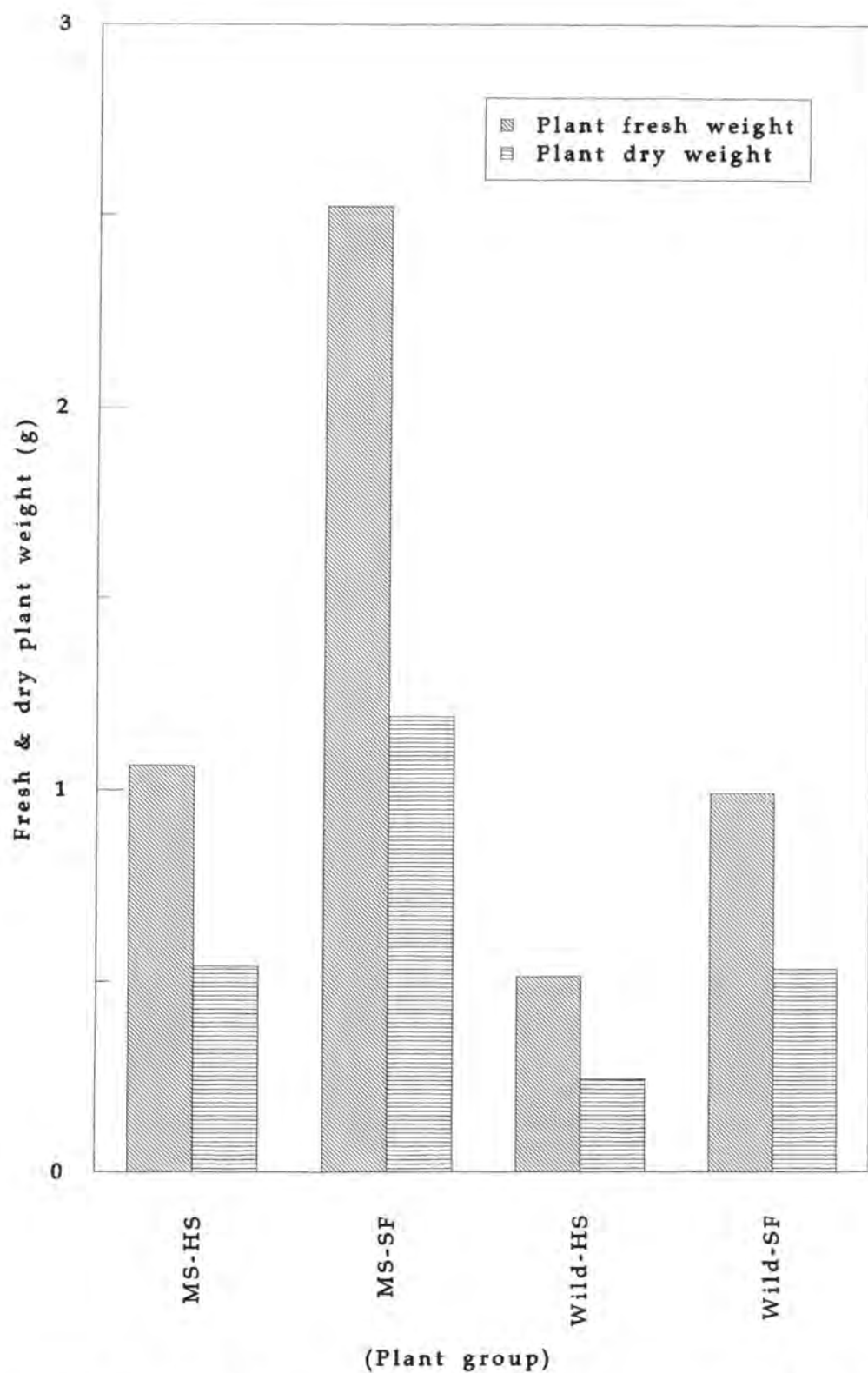


Fig. 7.9. Effect of zinc sulphate high stress (300 ppm) on the plant fresh and dry weight in *A. thaliana*.

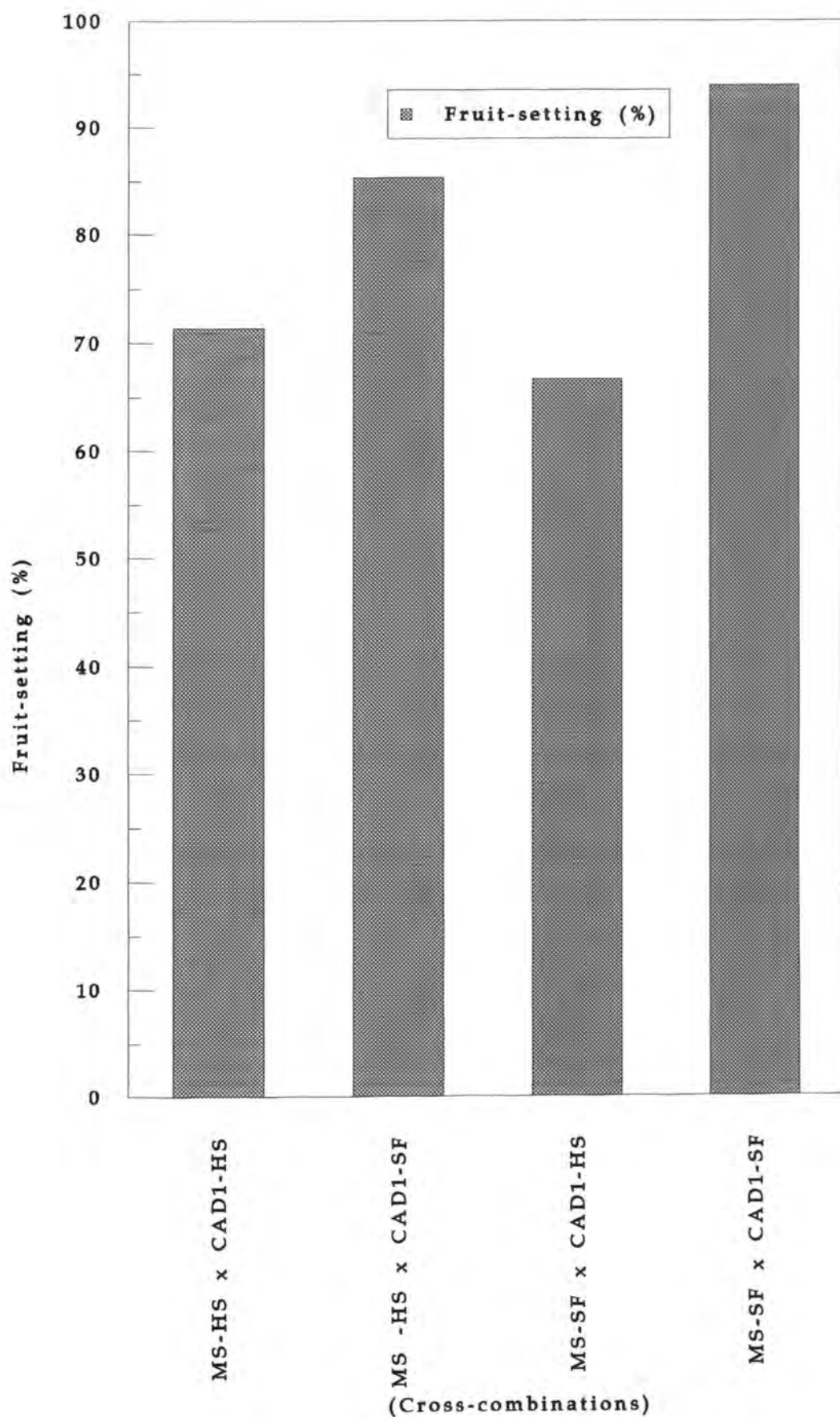


Fig. 7.10. Effect of cadmium sulphate high-stress (200 ppm) on the fruit-setting (%) of *A. thaliana* (CAD1).

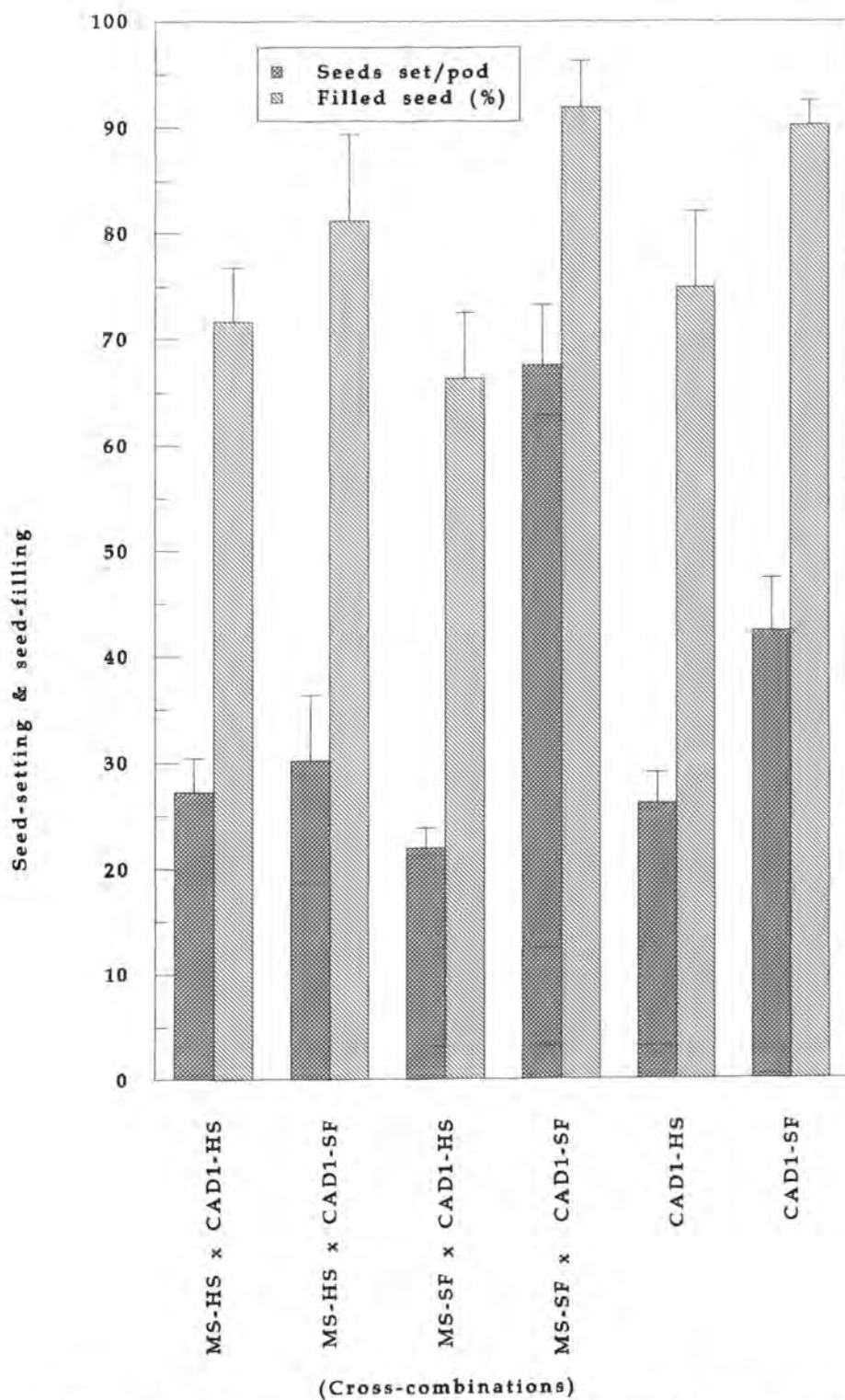


Fig. 7.11. Effect of cadmium sulphate high-stress (200 ppm) on the seed-setting and seed-filling of *A. thaliana* (CAD1).

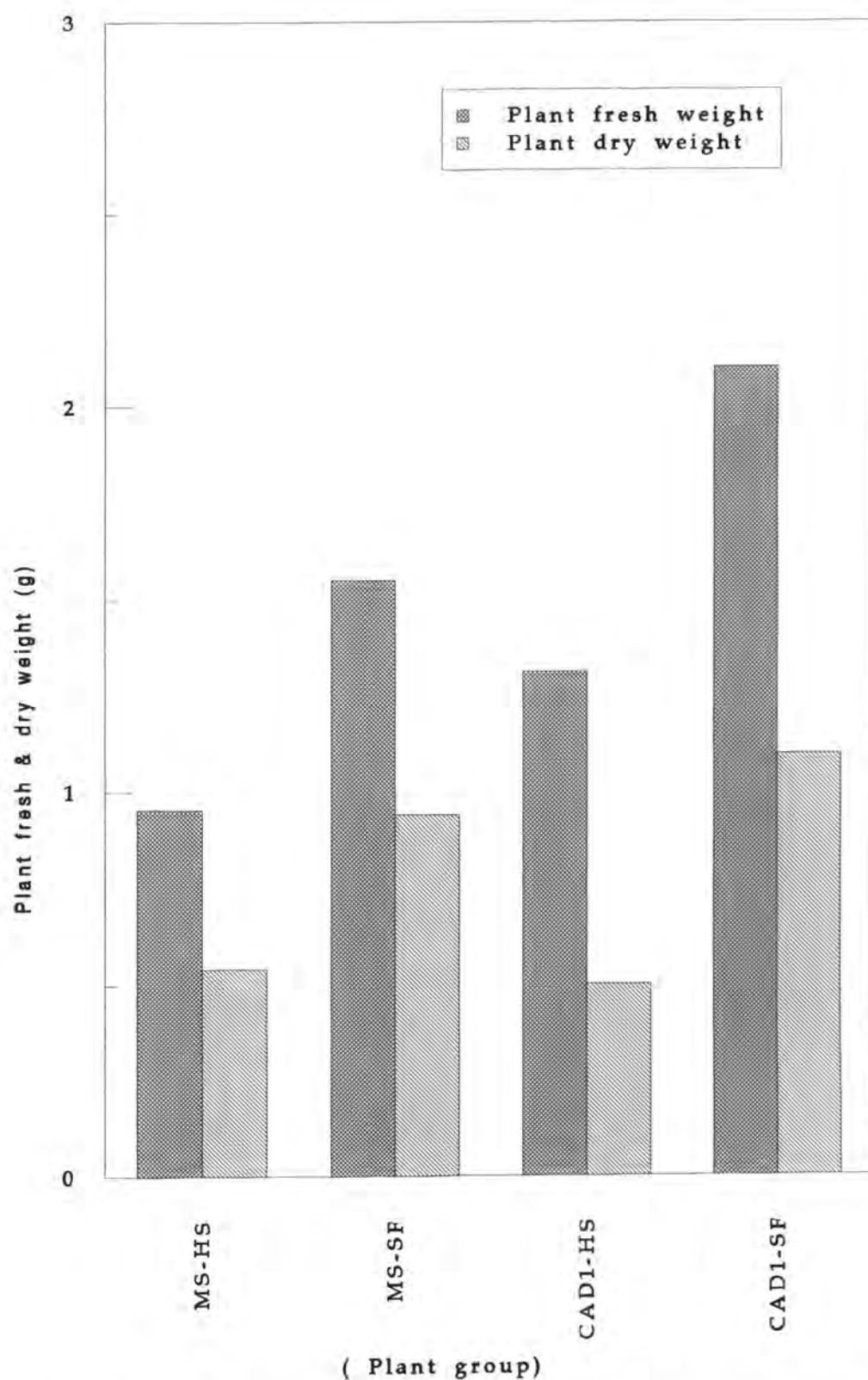


Fig. 7-12. Effect of cadmium sulphate high-stress (200 ppm) on plant fresh & dry weight in *A. thaliana* (CAD1).

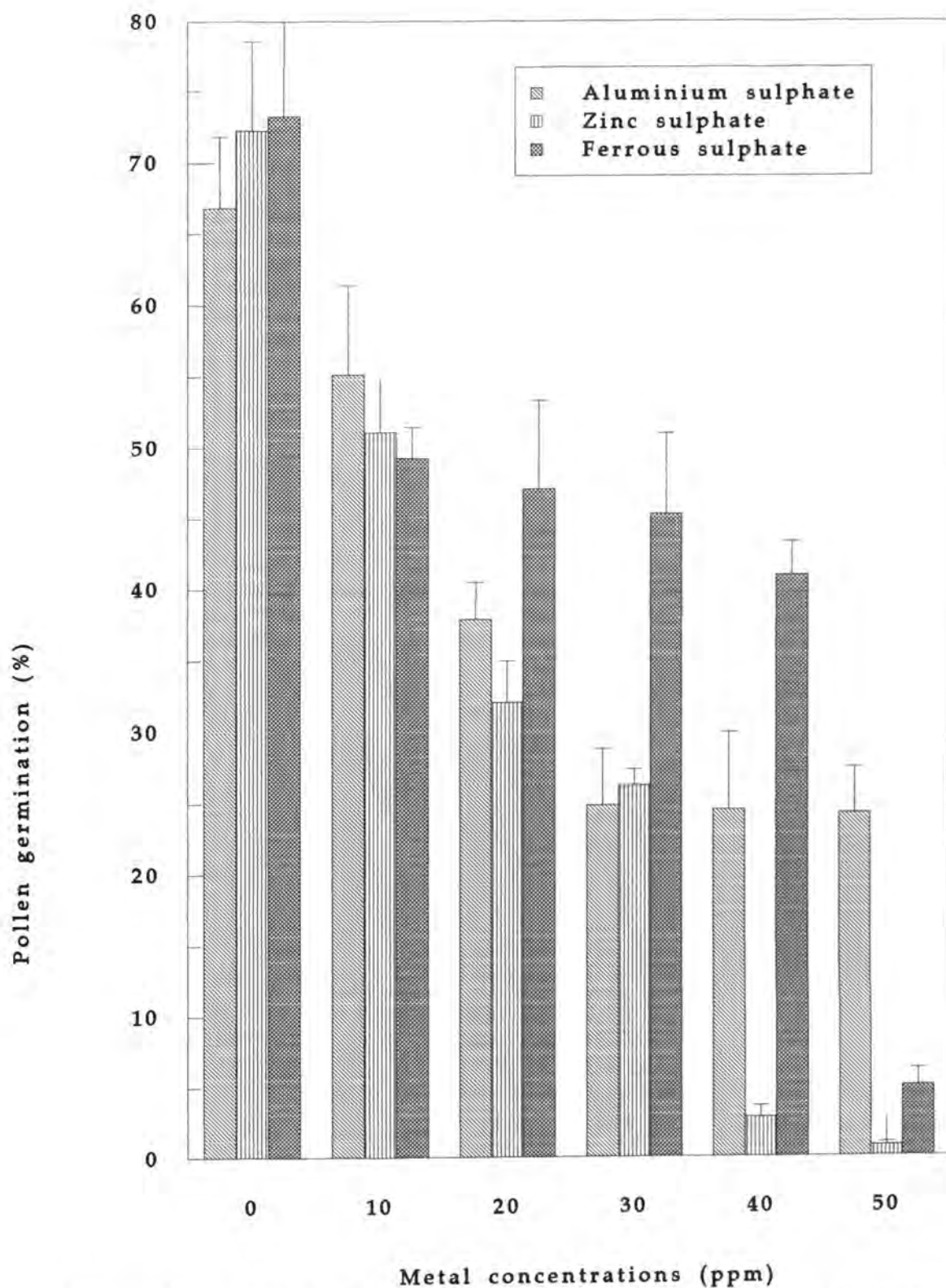


Fig. 7.13. Effect of Aluminium sulphate, zinc sulphate and ferrous sulphate levels (ppm) on the pollen germination of *Arabidopsis thaliana* (wild type).

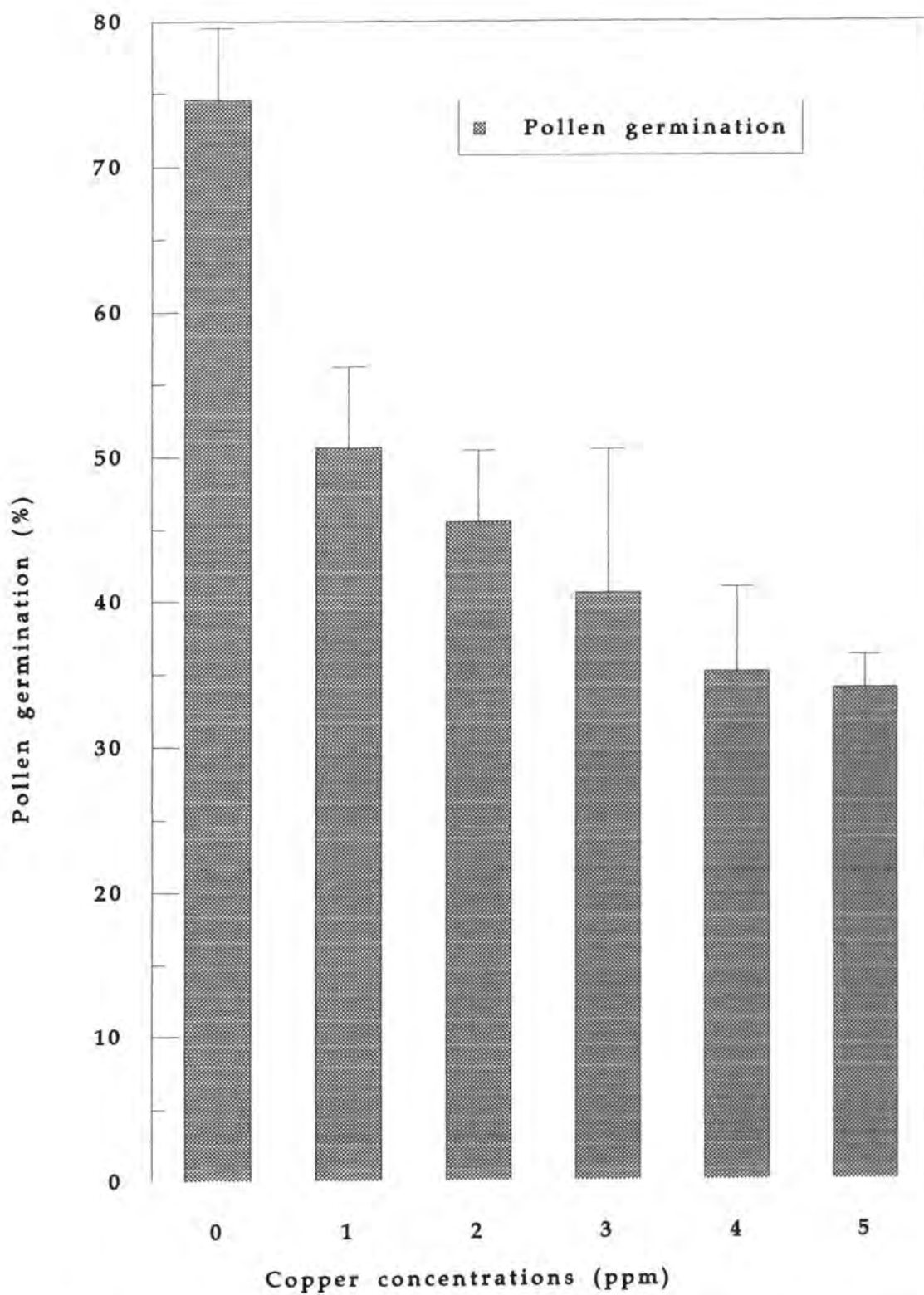


Fig. 7.14. Effect of copper sulphate concentrations on the pollen germination of *Arabidopsis thaliana* (wild type).

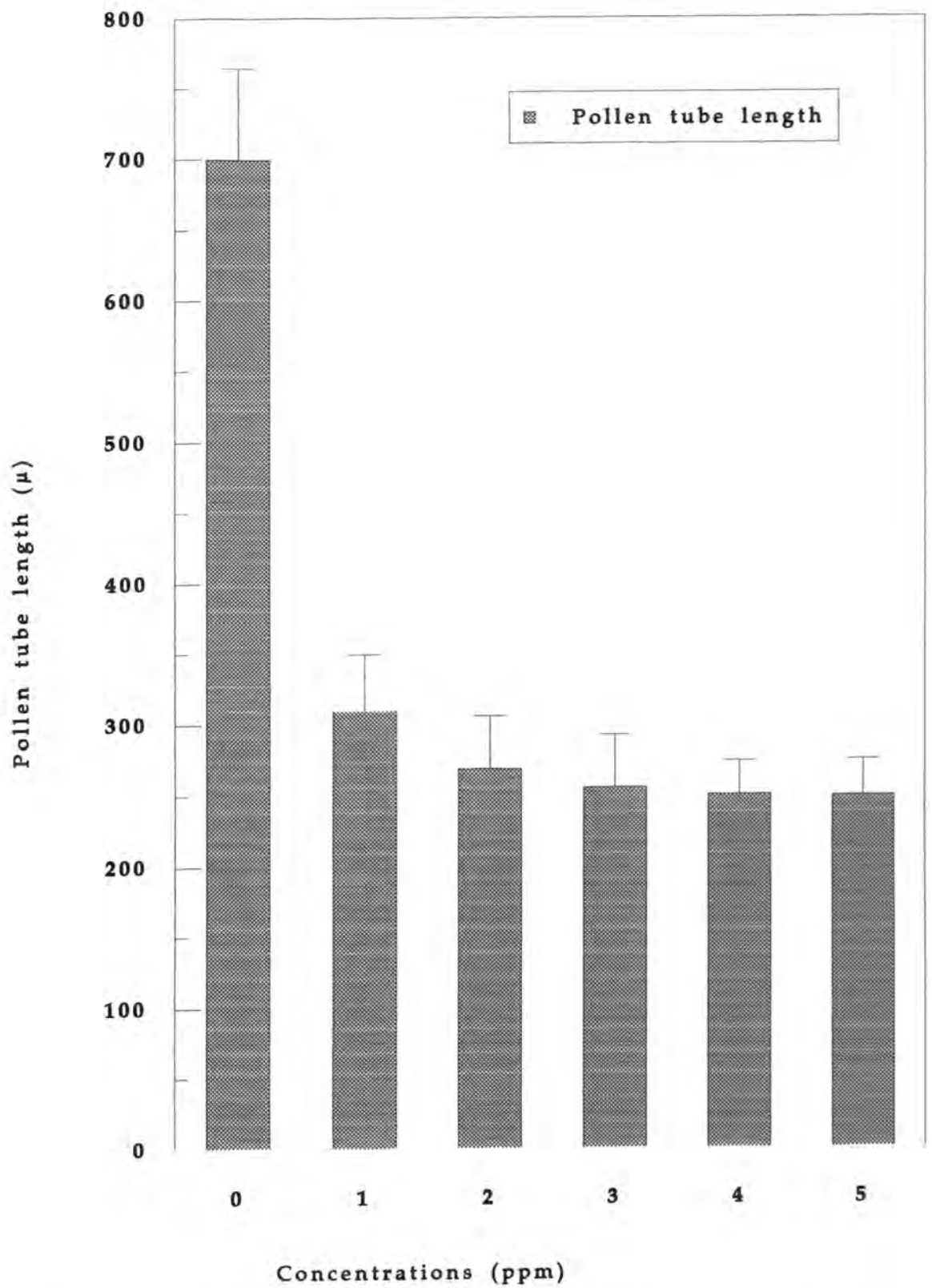


Fig. 7.15. Effect of copper sulphate concentrations on pollen tube length of *Arabidopsis thaliana* (wild type).

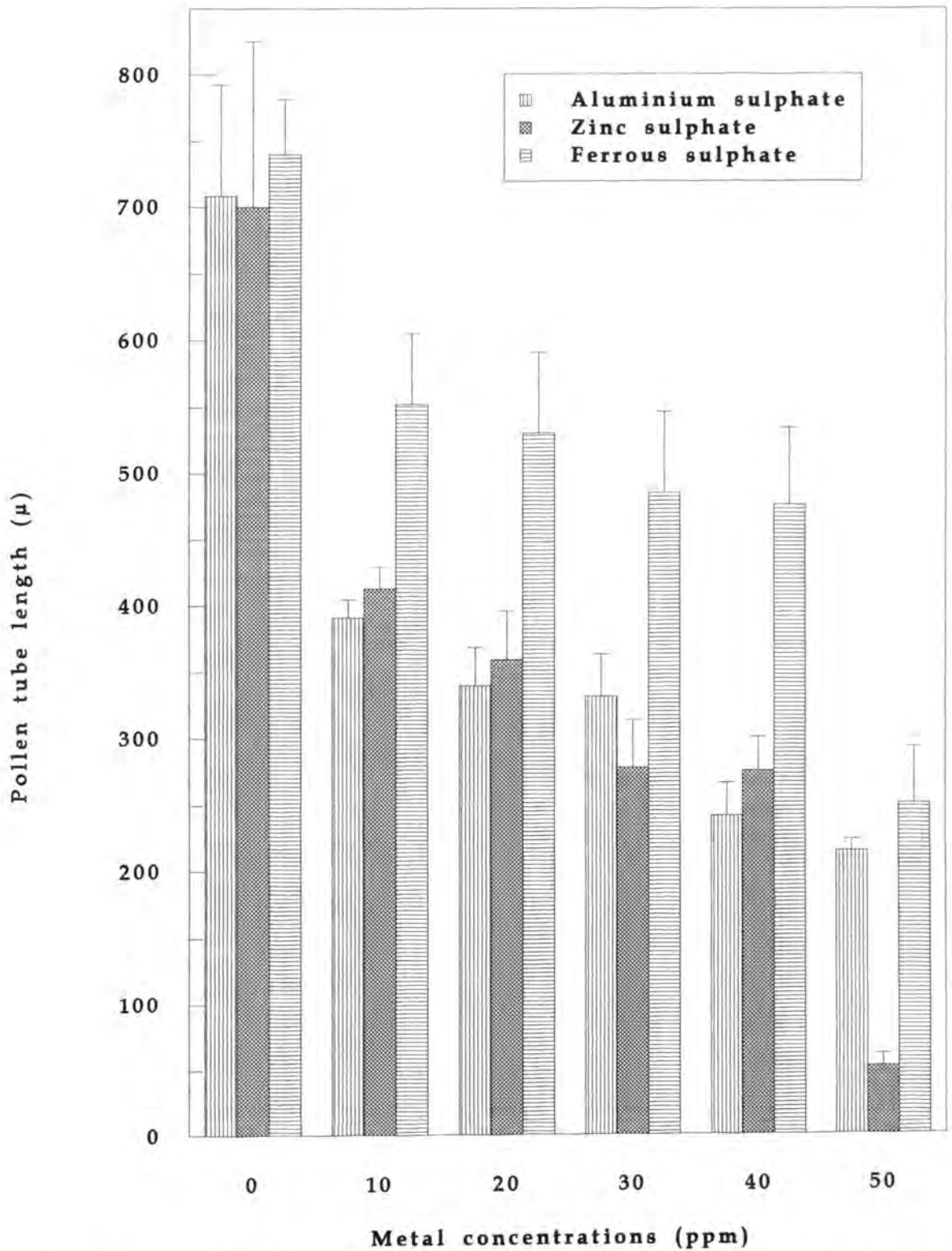


Fig. 7.16. Effect of aluminium sulphate, zinc sulphate and ferrous sulphate on the pollen tube length of *Arabidopsis thaliana* (wild type).

Chapter VIII

Anatomical changes caused by heavy metals in the stem, leaf and root of *Arabidopsis thaliana*

As mentioned in materials and methods, selected concentrations of $CdSO_4$, $Al_2(SO_4)_3$, $ZnSO_4$ and $CuSO_4$, were used to study the effects of these heavy metals on the anatomical structure of stem, leaf and root of *A. thaliana*. The detailed results are interpreted below:

8.1 Anatomical changes caused by cadmium

Increasing concentrations of $CdSO_4$ produced the following general anatomical changes in the stem, leaf and root:

8.1.1 Effect of cadmium on the stem anatomy of WT- genotype

The following anatomical changes (Plates-8.2-8.5) were caused by the $CdSO_4$ in the anatomy of stem tissues in comparison to the control.

- (a). An induction of irregular stem shape.
- (b). An increase in the cortex cell size.
- (c). Loss of chloroplasts in the cortical region.
- (d). A de-differentiation of the phloem and cambium.
- (e). Greater lignification of the xylem cells.
- (f). A disrupted pith region.

While quantifying the changes (Table-8.1) it was noticed that the stem diameter was increased by *Cd*-stress at all levels. The epidermal cell size was increased under lower stress regimes and was slightly decreased under the highest *Cd*-stress condition. The cortex cell diameter was increased under every *Cd*-stress regime. The cross section diameter of vascular bundles (CSDVB) was increased. The size of pith cells was larger under all *Cd*-stress conditions.

8.1.2 Effect of cadmium on the leaf anatomy of the WT-genotype

Cadmium caused the following structural changes (Plates-8.6-8.7) in the leaf tissues in comparison to the control:

- (a). An increase in the leaf thickness.
- (b). A reduction in the epidermal cell size.
- (c). A reduction in the palisade parenchyma cell size.
- (d). A reduction in the spongy mesophyll area.
- (e). A reduction in the size of intercellular spaces.
- (f). A reduction in the number of chloroplasts.

Numerical data (Table-8.2) revealed that the leaf thickness was enhanced under all *Cd*-stressed conditions. The size of the palisade cells increased under one stress level (150 ppm) and decreased under other stress levels compared with the control. The intercellular spaces were reduced in size under all *Cd*-stress levels.

8.1.3 Effect of cadmium on the root anatomy of WT-genotype

The *Cd*-stress regimes created (Plates-8.8-8.9) the following anomalies in the anatomy of root tissues in relation to the control.

- (a). Disruption of the cortical layers.
- (b). A lack of differentiation and increased lignification in the pericycle and the phloem.
- (c). An increase in diameter of the stele and the xylem cells.
- (d). Damage to the endodermis.

The data (Table-8.3) indicate that the stele diameter was slightly reduced under lower stress conditions, but was stimulated under elevated *Cd*-stress regimes. The xylem cell diameter was increased with the increasing *Cd*-stress, in relation to the control.

8.1.4 Effect of cadmium on the stem anatomy of the CAD1-genotype

Cadmium caused the following changes in the stem anatomy of the genotype CAD1 (Plates-8.10-8.13) in comparison to the control.

- (a). A severe change in the stem shape.
- (b). An increase in size of the epidermal cells.
- (c). A lack of differentiation of the cortical parenchyma, the vascular bundles and the pericycle, at disrupted sites within the stem.
- (d). A high level of lignification in the phloem fibres and resulting lack of differentiation of the phloem, the cambium and the xylem in certain vascular bundles.
- (e). Reduction in pith cell size.

The data recorded (Table-8.1) indicate that the stem diameter was reduced under all the *Cd*-stress regimes in relation to the control. The epidermal cell size was stimulated, while the cortex and the pith cell size was decreased. The CSDVB

reduced relative to the control, under all *Cd*-stress concentrations.

8.1.5 Effect of cadmium on the leaf anatomy of the CAD1-genotype

The *Cd*-stress caused changes in the leaf tissues (Plates-8.14-8.15) of genotype CAD1 in relation to the control.

- (a). An increase in size of the palisade parenchyma cells.
- (b). Loss of chloroplasts.
- (c). An increase in size of the intercellular spaces.
- (d). A reduction in the stomatal density.

The data (Table-8.2) show that the leaf thickness decreased under low cadmium levels and increased under the highest stress level. The palisade cell diameter decreased under low stress conditions and increased under elevated *Cd*-stress regimes. Size of the intercellular spaces was enhanced under all *Cd*-stress conditions.

8.1.6 Effect of cadmium on the root anatomy of the CAD1-genotype

The cadmium treatments (Plates-8.16-8.17) caused the following changes in the root tissues of genotype CAD1 in comparison to the control:

- (a). Disruption of the cortical region and the endodermis.
- (b). A lack of differentiation of the phloem and the pericycle.
- (c). An increase in the xylem cell size, with more lignification.

The data depicted in Table-8.3 indicate that the stele diameter of the root decreased under low concentrations of *Cd*-stress and increased under the highest stress level. Xylem cell diameter was generally stimulated under increasing concentrations of *Cd*-stress.

8.2 Effect of aluminium sulphate on the anatomy of the stem, leaf and root

The aluminium stress conditions created the following anatomical changes in the tissues of stem, leaf and root.

8.2.1 Effect of aluminium sulphate on the stem anatomy

The seedlings treated with aluminium sulphate showed (Plate-8.19-8.21) the following general anatomical changes in the stem tissues in relation to the control.

- (a). An increase in diameter of the cortex cells and cortical cell layers.
- (b). More lignification in vascular bundles.

The data (Table-8.1) show that the epidermal cell size was reduced under low *Al*-stress levels, but enhanced under the highest stress level. The cortex cell size declined slightly under the 300 ppm level of *Al*-stress, but increased under remaining *Al*-stress regimes. The CSDVB was stimulated under all *Al*-stress concentrations. The pith cell size and the stem diameter increased at the lowest stress concentration, but were reduced under increasing *Al*-stress regimes.

8.2.2 Effect of aluminium sulphate on the leaf anatomy

The following changes were recorded (Plate-8.22) in the leaf anatomy by the $Al_2(SO_4)_3$ treatments in comparison to the control.

- (a). A reduction in cell size of the palisade parenchyma and the spongy mesophyll.
- (b). A reduction in the intercellular spaces.
- (c). An increase in the chloroplast content.
- (d). A reduction in the adaxial and the abaxial epidermal cell size.

The results (Table-8.2) indicate that the palisade cell size was stimulated under the lowest *Al*-stress and decreased under elevated concentrations. The intercellular spaces were generally smaller than the control under all *Al*-stress regimes. The leaf thickness was slightly enhanced under 300 ppm *Al*-stress, but declined under other stress regimes.

8.2.3 Effect of aluminium sulphate on the root anatomy

The following changes (Plate-8.23) were created by the $Al_2(SO_4)_3$ stress regimes in the root tissues in comparison to the control:

- (a). A disruption of the cortical region.
- (b). A disruption of the endodermis.
- (c). An increase in size of the xylem cells.
- (d). An increase in the stele diameter.
- (e). A lignification in the stelar region.

The data (Table-8.3) show that the stele diameter decreased in the lowest *Al*-stress concentration and was stimulated under increasing *Al*-stress concentrations.

8.3 Effect of zinc sulphate on the stem, leaf and root anatomy

Zinc sulphate created the following anatomical changes in the stem, leaf and root in the plants treated with different $ZnSO_4$ -stress regimes, in comparison to the control.

8.3.1 Effect of zinc sulphate on the stem anatomy

The following general changes were noticed (Plate-8.24-8.26) in the anatomy

of the stem tissues, created by the various concentrations of $ZnSO_4$ -stress in comparison to the control:

- (a). An increase in the stem diameter.
- (b). An increase in size of the cortical cells.
- (c). A reduction in the chloroplast density in the cortical cells.
- (d). An increased lignification in the pericycle and the xylem.
- (e). An increase in size of the pith cells.
- (f). An increase in the CSDVB.
- (g). A slight change in the stem shape.

The data (Table-8.1) show that the stem diameter was larger. The CSDVB increased under stress conditions. Size of the cortex and pith cells was increased under all *Zn*-stress regimes in relation to the control, while the epidermal cell size declined under the lowest stress level and was stimulated under increasing stress regimes.

8.3.2 Effect of zinc sulphate on the leaf anatomy

The *Zn*-stress regimes (Plate-8.27) caused the following changes in the leaf tissues in comparison to the control:

- (a). An increase in the size of the palisade parenchyma and the spongy mesophyll cells.
- (b). An increase in the chloroplast density of mesophyll cells.
- (c). An increase in size of the epidermal cells on either side of the leaf.
- (d). A reduction in the stomata number on the adaxial side of the leaf.

The data (Table-8.2) indicate that leaf thickness was nominally reduced under the highest *Zn*-stress level, but was stimulated under other stress conditions. The palisade parenchyma cell size increased under all *Zn*-stress regimes. The size of the intercellular spaces increased under the highest stress condition and declined under other stress regimes.

8.3.3 Effect of zinc sulphate on the root anatomy

The conditions of *ZnSO*₄ (Plate-8.28) created the following changes in the root tissues in comparison to the control:

- (a). A reduction in the xylem & cortex cell size and the stele diameter.
- (b). A lignification in the pericycle.
- (c). A reduction in the endodermal cell size.

The data (Table-8.3) show that the cortex and the xylem cell size and the stele diameter were stimulated in relation to the control under all *Zn*-stress regimes. It is remarkable to note that under all heavy metal stress conditions the cortex was generally disrupted but exceptionally under *Zn*-stress regimes it remained intact.

8.4 Effect of copper sulphate on the stem anatomy

The following general anatomical changes were noted in the stem, leaf and root in *CuSO*₄-treated seedlings:

8.4.1 Effect of copper sulphate on the stem anatomy

The *CuSO*₄-stress regimes induced the following anatomical changes (Plate-8.29-8.31) in the stem tissues, relative to the control:

- (a). A slight change in the stem shape.
- (b). An increase in the pericycle and the pith cell size because of their deterioration.
- (c). Disorganization of the pericycle and the pith.
- (d). A lignification in the xylem cells.
- (e). A reduction in the chloroplast number.

The data (Table-8.1) indicate that the cortex, pith and the CSDVB all increased in size under all $CuSO_4$ -stress regimes. The epidermal cell size increased under the highest stress concentration and decreased under other stress regimes. The stem diameter decreased under the lowest stress level and increased under increasing levels of stress in comparison to the control.

8.4.2 Effect of copper sulphate on the leaf anatomy

The following anatomical changes (Plate- 8.32) were observed in the leaf in comparison to the control when the seedlings were subjected to the $CuSO_4$ -stress conditions:

- (a). An increase in the palisade parenchyma cell size.
- (b). A reduction in the intercellular spaces and the leaf thickness.
- (c). An increase in chloroplast number under the lowest-stress and a decrease under elevated levels.

The results (Table-8.2) demonstrate that the palisade parenchyma cell size was enhanced under every Cu -stress regime. The size of the intercellular spaces and the leaf thickness decreased under every Cu -stress condition.

8.4.3 Effect of copper sulphate on the root anatomy

The following effects were recorded (Plate-8.33) on the anatomy of root tissues caused by the *Cu*-stress regimes in comparison to the control:

- (a). A disruption of the cortical region and the endodermis.
- (b). Relatively more lignification in the pericycle and the xylem cells.
- (c). An increase in size of the xylem cells.

The results (Table-8.3) indicate that the cortex cell size generally decreased, while the xylem cell size increased under all *Cu*-stress conditions. The stele diameter was reduced under the lowest stress condition, and increased under increasing *Cu*-stress regimes.

Table-8.1: The stem diameter, cell size of cortex & pith cell and cross sectional diameter of vascular bundle (CSDVB) (μm) of stem of *A. thaliana* raised under different concentrations of heavy metal regimes.

Heavy metal (ppm)	Stem diameter (μm)	Epidermis cell size (μm)	Cortex cell size (μm)	CSDVB (μm)	Pith cell size (μm)
<i>CdSO₄</i> (WT)					
100	424.00 \pm 24.00 (+21.14)	8.70 \pm 1.27 (+19.17)	12.38 \pm 1.55 (+54.50)	95.00 \pm 8.66 (+21.79)	37.50 \pm 9.31 (+191.60)
150	366.00 \pm 106.00 (+4.57)	7.60 \pm 1.11 (+4.10)	16.40 \pm 7.51 (+105.00)	ndv -	40.00 \pm 17.26 (+211.04)
200	384.00 \pm 64.00 (+9.71)	7.20 \pm 1.08 (-1.37)	12.30 \pm 2.10 (+53.75)	120.00 \pm 16.33 (+53.85)	14.67 \pm 6.79 (+14.07)
<i>CdSO₄</i> (CAD1)					
100	490.00 \pm 10.00 (-14.03)	10.10 \pm 1.76 (+40.22)	10.60 \pm 1.74 (-1.85)	110.00 \pm 17.32 (-3.17)	26.00 \pm 2.44 (-20.00)
150	400.00 \pm 120.83 (-29.82)	7.90 \pm 0.94 (+9.72)	6.40 \pm 0.49 (-59.26)	78.00 \pm 14.56 (-31.34)	21.20 \pm 4.66 (-34.77)
200	368.00 \pm 67.49 (-35.44)	7.30 \pm 1.10 (+1.39)	6.00 \pm 0.45 (-44.44)	104.00 \pm 4.00 (-8.45)	14.00 \pm 2.00 (-56.92)
Control (CAD1)	570.00 \pm 2.50	7.20 \pm 1.33	10.80 \pm 1.99	113.60 \pm 14.22	32.50 \pm 8.05
<i>Al₂(SO₄)₃</i>					
200	368.00 \pm 12.00 (+5.14)	6.7 \pm 0.78 (-8.22)	12.50 \pm 2.80 (+56.25)	86.28 \pm 18.50 (+10.61)	18.00 \pm 5.24 (+39.75)
300	324.00 \pm 4.00 (-7.42)	6.10 \pm 1.13 (-16.44)	7.70 \pm 2.10 (-3.75)	99.00 \pm 4.36 (+26.92)	12.40 \pm 1.74 (-3.72)
500	316.00 \pm 4.00 (-9.71)	7.80 \pm 2.27 (+6.84)	9.70 \pm 2.32 (+21.25)	83.33 \pm 11.17 (+6.83)	12.60 \pm 2.20 (-2.17)

Table-8.1 (continued): The stem diameter, cell size of coretex & pith cell and cross sectional diameter of vascular bundle (CSDVB) (μm) of stem of *A. thaliana* raised under different concentrations of heavy metal regimes.

<i>ZnSO₄</i>					
100	546.00 \pm 6.00 (+56.00)	6.20 \pm 0.14 (-15.07)	10.25 \pm 2.89 (+28.12)	153.33 \pm 18.86 (+96.58)	33.50 \pm 5.17 (+160.49)
200	420.00 \pm 20.00 (+20.00)	9.30 \pm 2.45 (+27.39)	19.30 \pm 5.66 (141.25)	97.33 \pm 3.77 (+24.78)	ndv -
300	436.41 \pm 44.00 (+24.69)	9.50 \pm 1.56 (+30.14)	12.80 \pm 3.19 (+60.00)	96.80 \pm 15.47 (+24.10)	30.40 \pm 8.33 (+136.39)
<i>CuSO₄</i>					
10	316.00 \pm 4.00 (-9.71)	6.00 \pm 1.33 (-17.81)	10.20 \pm 3.03 (+27.50)	103.00 \pm 10.34 (+32.05)	29.43 \pm 5.73 (+129.92)
15	406.00 \pm 26.00 (+16.00)	6.60 \pm 1.36 (-9.58)	9.20 \pm 1.40 (+15.00)	103.33 \pm 7.45 (+32.47)	29.14 \pm 7.91 (+127.66)
20	478.00 \pm 34.00 (+36.57)	9.50 \pm 2.46 (+30.14)	15.70 \pm 1.73 (+96.25)	88.80 \pm 6.88 (+13.85)	44.00 \pm 15.39 (+243.75)
Control	350.00 \pm 10.00	7.30 \pm 0.78	8.00 \pm 1.85	78.00 \pm 3.46	12.86 \pm 1.28

ndv denotes non-determined values

Table-8.2: The leaf thickness & palisade cell and intercellular space size (μm) of leaf of *A. thaliana* raised under different concentrations of heavy metal regimes.

Heavy metal (ppm)	Leaf thickness (μm)	Palisade cell size (μm)	Intercellular space cell size (μm)
<i>CdSO₄</i> (WT)			
100	86.67 \pm 2.49 (+9.51)	10.70 \pm 2.050 (-20.39)	9.00 \pm 1.00 (-51.60)
150	79.55 \pm 8.26 (+0.52)	15.90 \pm 3.36 (+18.30)	16.60 \pm 6.02 (-10.75)
200	84.67 \pm 3.59 (+6.99)	10.43 \pm 0.90 (-22.39)	7.33 \pm 1.25 (-60.59)
<i>CdSO₄</i> (CAD1)			
100	56.40 \pm 5.28 (-27.69)	9.80 \pm 1.54 (-8.15)	14.80 \pm 4.28 (+53.05)
150	58.80 \pm 8.16 (-24.61)	12.10 \pm 2.30 (+13.40)	16.40 \pm 3.88 (+69.60)
200	89.60 \pm 3.67 (+14.87)	12.33 \pm 2.90 (+15.56)	12.33 \pm 5.18 (+27.51)
Control (CAD1)	78.00 \pm 6.93	10.67 \pm 2.05	9.67 \pm 0.82
<i>Al₂(SO₄)₃</i>			
200	75.33 \pm 4.00 (-4.81)	16.60 \pm 4.26 (+23.51)	15.00 \pm 3.26 (-19.35)
300	81.00 \pm 10.25 (+2.35)	12.40 \pm 2.53 (-7.73)	12.60 \pm 2.15 (-32.25)
500	50.00 \pm 4.56 (-36.82)	3.90 \pm 0.70 (-70.98)	ndv -

Table-8.2 (continued): The leaf thickness & palisade cell and intercellular space size (μm) of leaf of *A. thaliana* raised under different concentrations of heavy metal regimes.

<i>ZnSO</i> ₄			
100	82.40 ±8.98 (+4.12)	15.00 ±1.90 (+11.61)	14.40±3.38 (-22.58)
200	90.80 ±6.27 (+14.73)	24.70 ±9.13 (+83.78)	17.60 ±3.38 (-5.38)
300	79.20 ±5.74 (-0.07)	26.50 ±11.59 (+97.17)	19.50 ±5.72 (+4.84)
<i>CuSO</i> ₄			
10	78.00 ±7.90 (-1.44)	15.50 ±2.38 (+15.33)	12.20 ±2.92 (-34.40)
15	57.20 ±2.40 (-27.72)	16.40 ±5.75 (+22.02)	14.60 ±6.37 (-21.50)
20	59.67 ±14.11 (-24.60)	15.50 ±3.17 (+15.33)	12.20 ±2.78 (-34.40)
Control	79.14 ±6.13	13.44 ±3.89	18.60 ±4.22

ndv denotes non-determined values

Table-8.3: The stele diameter & cortex and xylem cell size (μm) of root of *A. thaliana* (WT) raised under different heavy metal regimes.

Heavy metal (ppm)	Stele diameter (μm)	Xylem cell size (μm)	Cortex cell size (μm)
<i>CdSO₄</i> (WT)			
100	59.00 \pm 1.00 (-1.67)	7.75 \pm 1.48 (+45.40)	ndv -
150	83.00 \pm 3.00 (+38.33)	8.80 \pm 1.94 (+65.10)	ndv -
200	104.00 \pm 30.00 (+73.33)	11.25 \pm 1.78 (+111.07)	ndv -
<i>CdSO₄</i> (CAD1)			
100	24.00 \pm 6.00 (-60.00)	6.33 \pm 2.05 (-23.27)	ndv -
150	40.00 \pm 10.00 (-33.33)	9.75 \pm 1.92 (+18.18)	ndv -
200	68.00 \pm 2.00 (+13.33)	9.00 \pm 0.63 (+9.09)	ndv -
Control	60.00 \pm 10.00	8.25 \pm 2.49	ndv
<i>Al₂(SO₄)₃</i>			
200	31.00 \pm 3.00 (-48.33)	5.50 \pm 0.50 (+3.19)	ndv -
300	79.00 \pm 3.00 (+31.66)	5.75 \pm 1.09 (+7.88)	ndv -
500	142.00 \pm 28.00 (+136.67)	9.67 \pm 2.36 (+81.42)	ndv -

Table-8.3 (continued): The stele diameter & cortex and xylem cell size (μm) of root of *A. thaliana* (WT) raised under different heavy metal regimes.

<i>ZnSO</i> ₄			
100	21.00 \pm 5.00 (-65.00)	3.00 \pm 0.00 (-43.71)	12.00 \pm 2.19 (-55.55)
200	29.20 \pm 5.00 (-51.33)	3.00 \pm 0.00 (-43.71)	18.37 \pm 3.67 (-31.96)
300	29.10 \pm 51.00 (-51.50)	4.00 \pm 0.00 (-24.95)	16.80 \pm 5.32 (-37.78)
<i>CuSO</i> ₄			
10	34.00 \pm 2.00 (-43.33)	ndv -	10.50 \pm 2.76 (-61.11)
15	136.00 \pm 28.00 (+126.67)	9.80 \pm 3.54 (+38.86)	25.40 \pm 8.52 (-5.92)
20	162.00 \pm 58.00 (+170.00)	16.40 \pm 2.73 (+207.69)	ndv -
Control	60.00 \pm 20.00	5.33 \pm 1.10	27.00 \pm 5.23

ndv denotes non-determined values

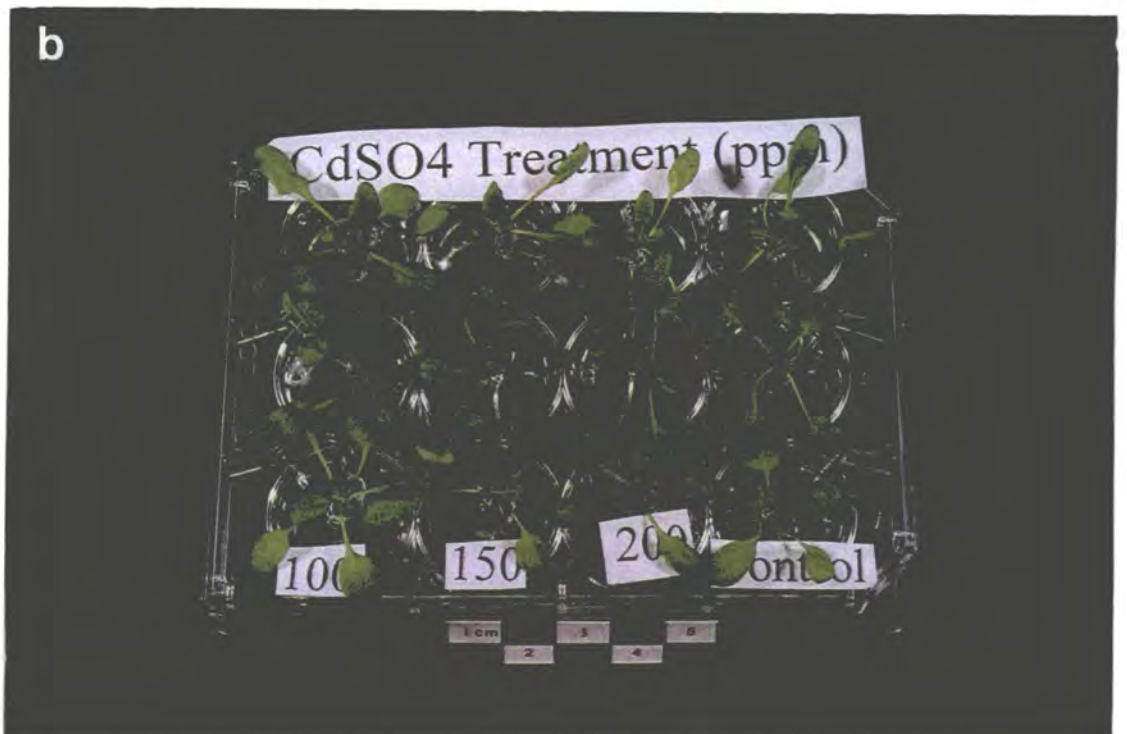
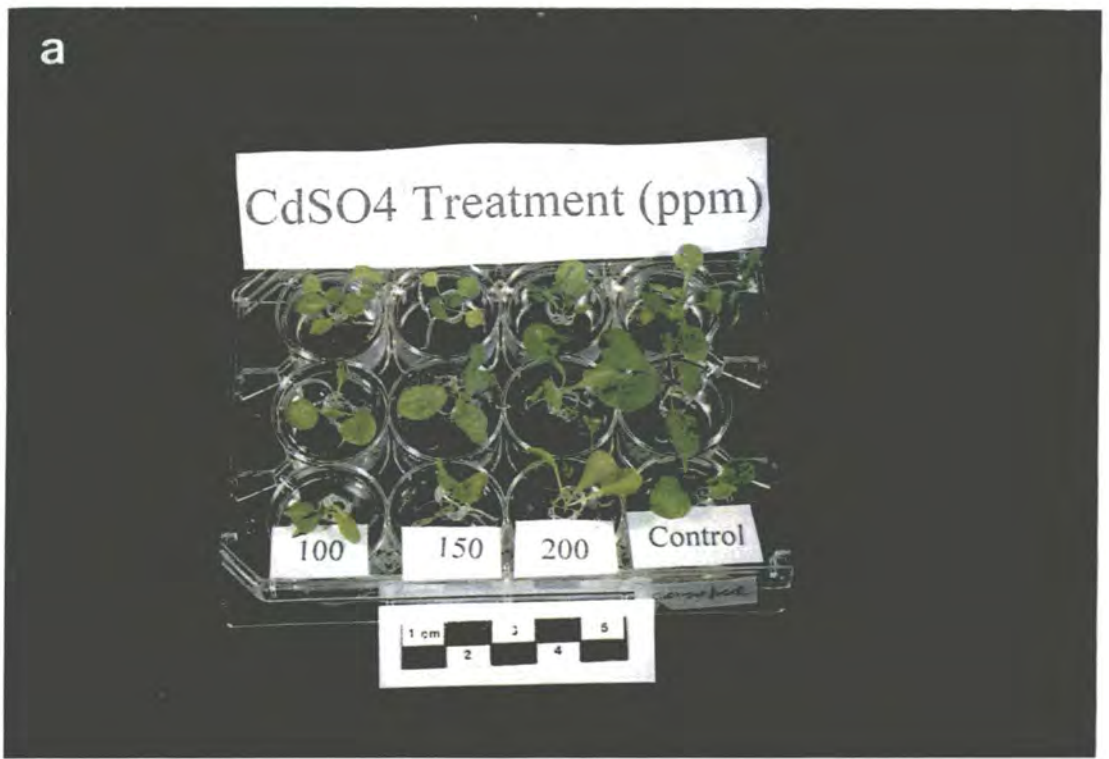


Plate 8.1 a-b. Seedlings of *Arabidopsis thaliana* (WT), growing under CdSO₄- stress regimes (A = WT, B = CAD1) and without stress conditions (control), for observing the anatomical changes in the stem, leaf and root tissues. WT denotes wild type.

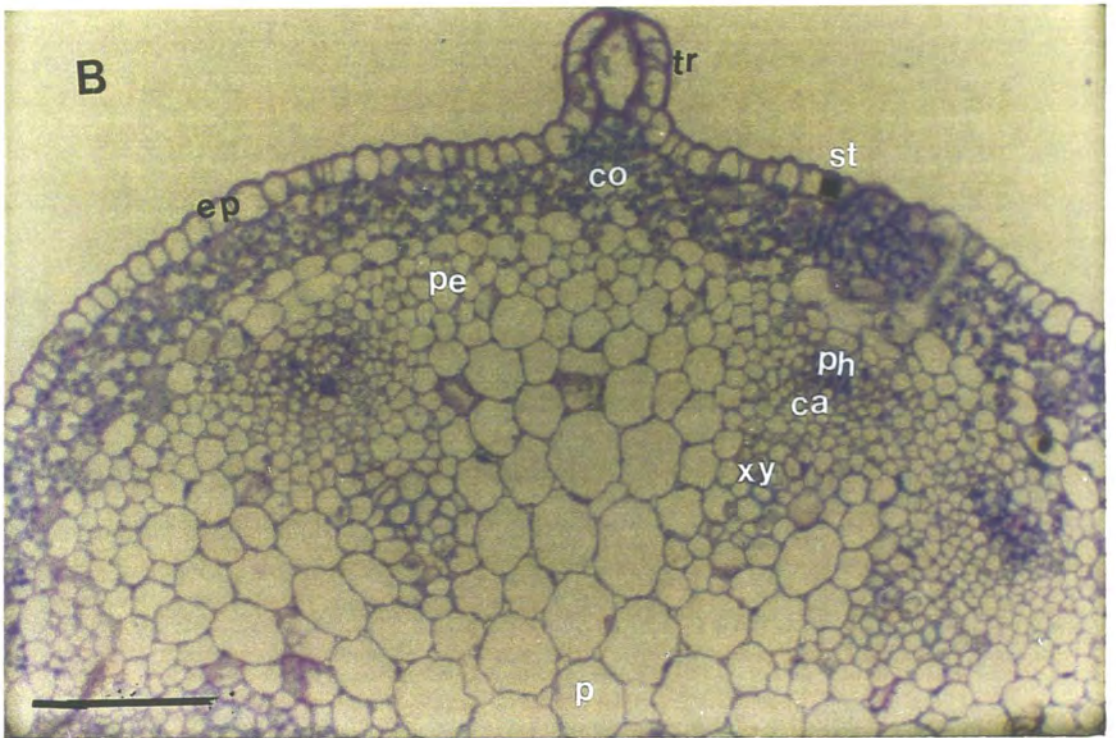
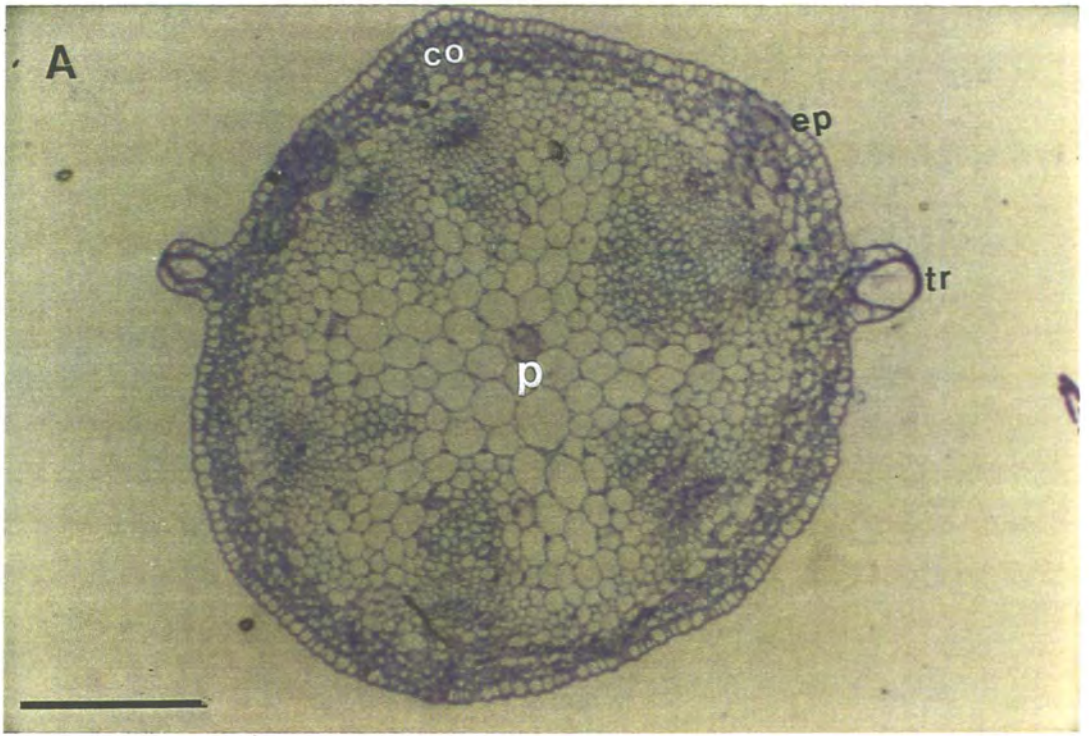


Plate 8.2 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised in the absence of CdSO_4 -stress conditions (control).

Plate 8.2 (B). Notice large chloroplast number in cortical region and non-lignified pericycle. ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm)

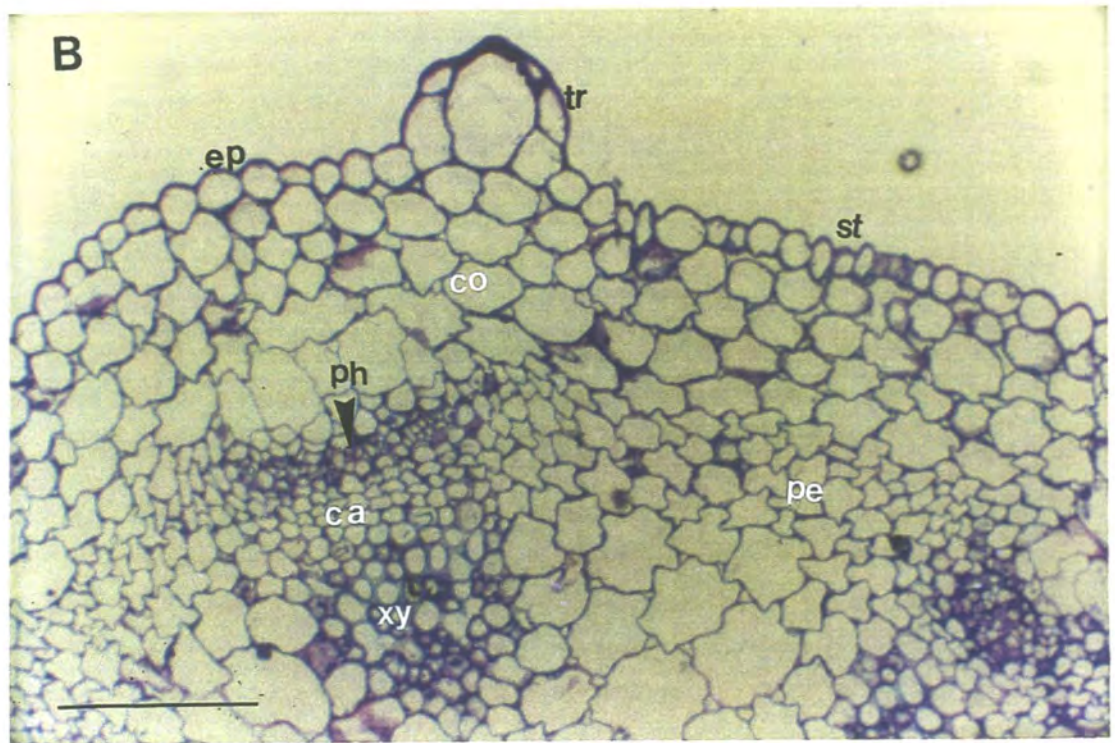
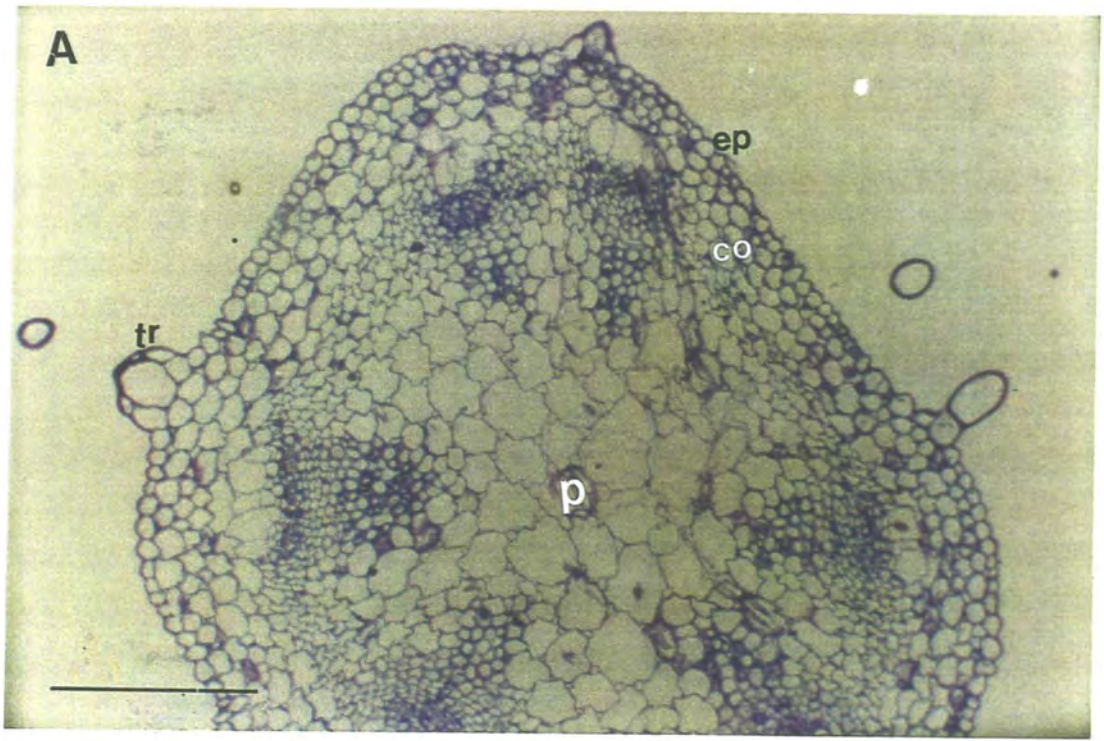


Plate 8.3 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under CdSO₄-stress conditions (100 ppm).

Plate 8.3 (A). Notice irregular shape of the stem.

Plate 8.3 (B) . Notice larger cortex cells devoid of chloroplasts, disturbed and irregular pericycle cells & more lignified phloem and xylem.

tr, trichome; ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm, B = 50 μm).

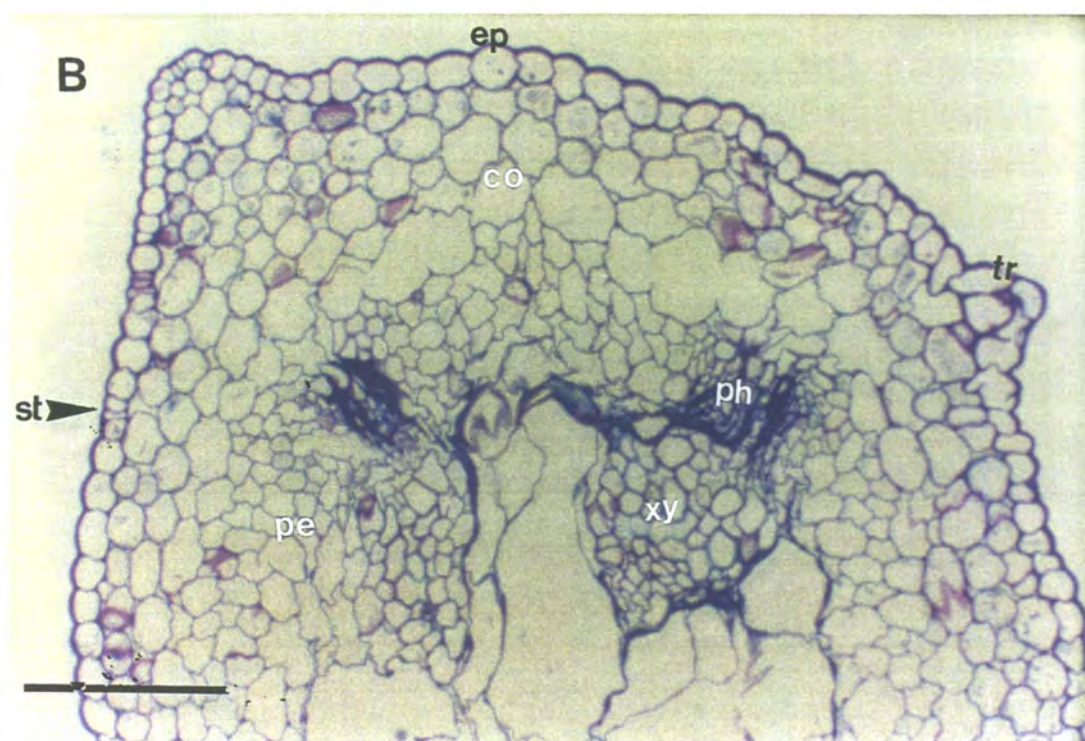
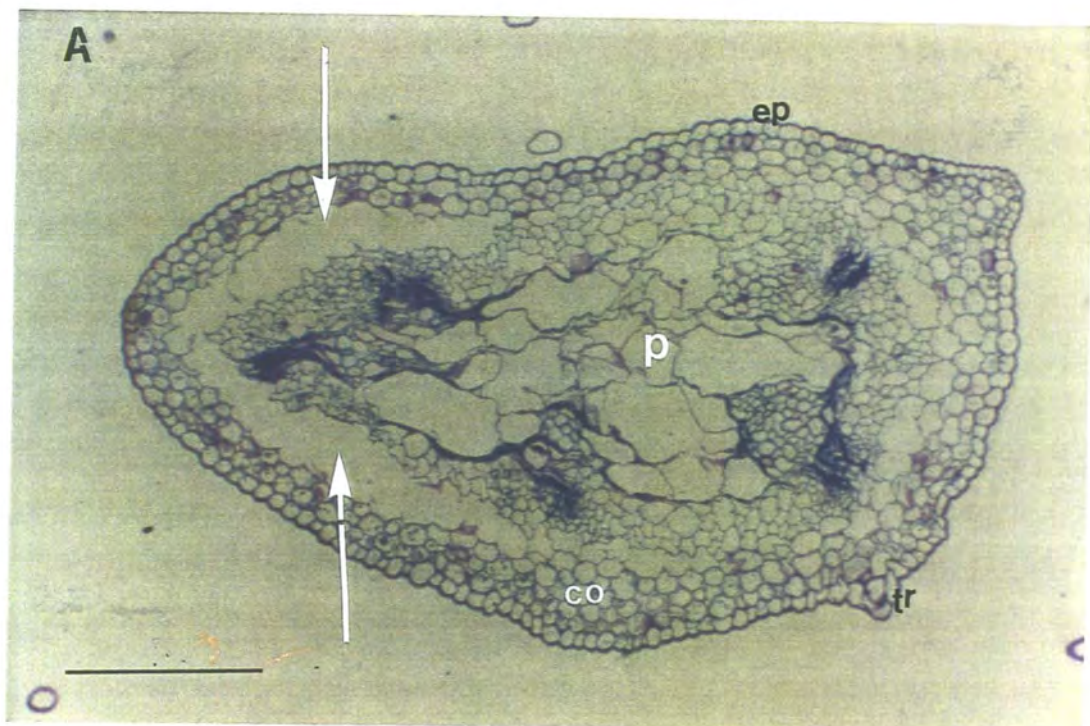


Plate 8.4 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under CdSO_4 -stress conditions (150 ppm).

Plate 8.4 (A). Notice irregular shape and disrupted regions (arrowed) of the stem.

Plate 8.4 (B). Notice larger cortex cells devoid of chloroplasts, undifferentiated phloem and cambium, lignified xylem and disrupted pith.

tr, trichome; ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm).

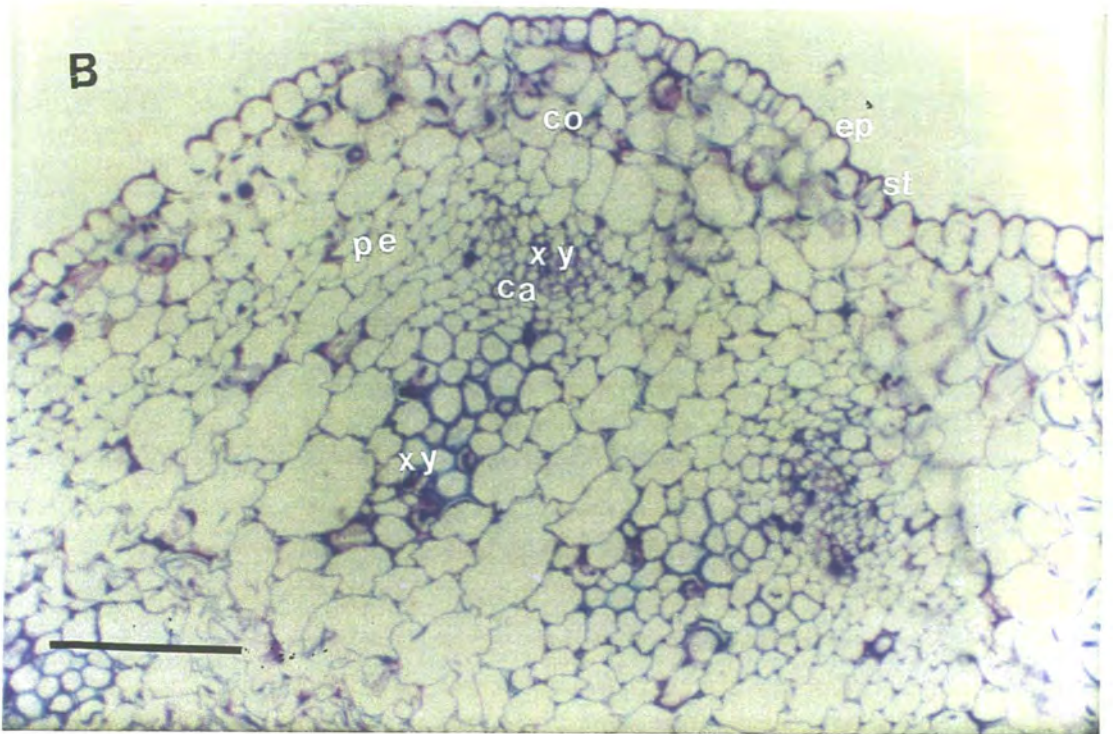
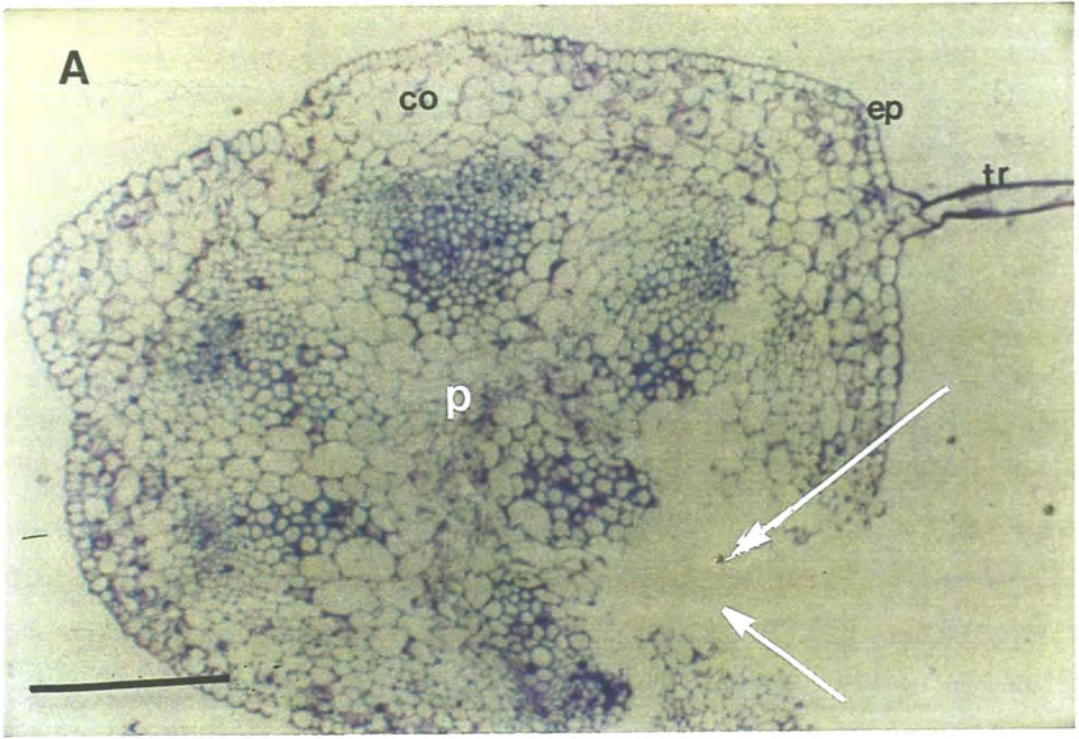


Plate 8.5 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under CdSO₄-stress conditions (200 ppm).

Plate 8.5 (A). Notice irregular shape and disrupted region (arrowed) of the stem.

Plate 8.5 (B). Notice larger cortex cells devoid of chloroplasts, more lignified xylem and very small pith cells.

tr, trichome; ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm, B = 50 μm).

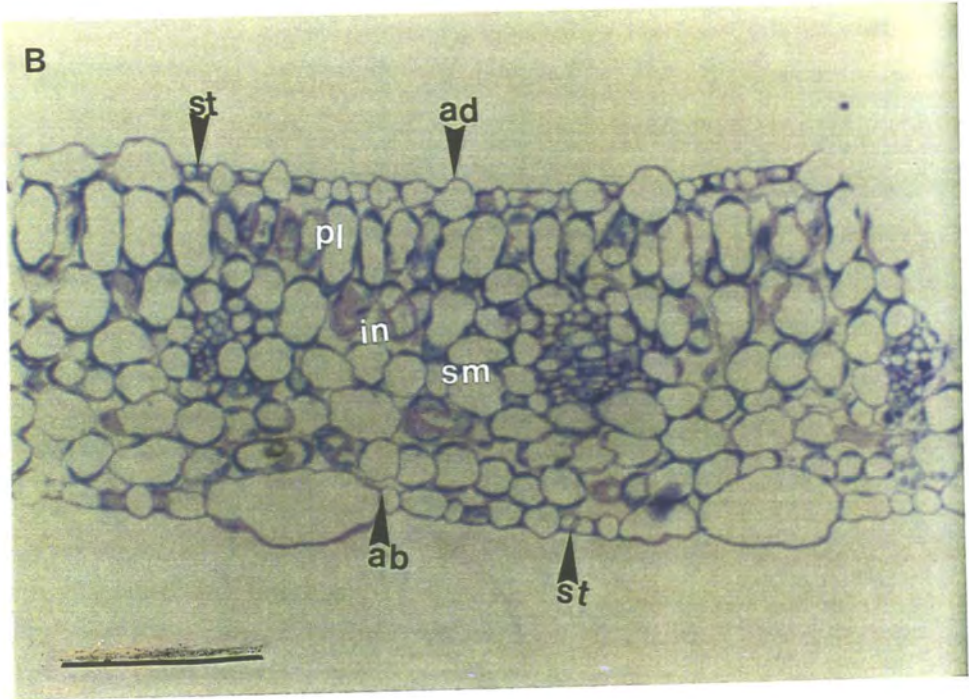
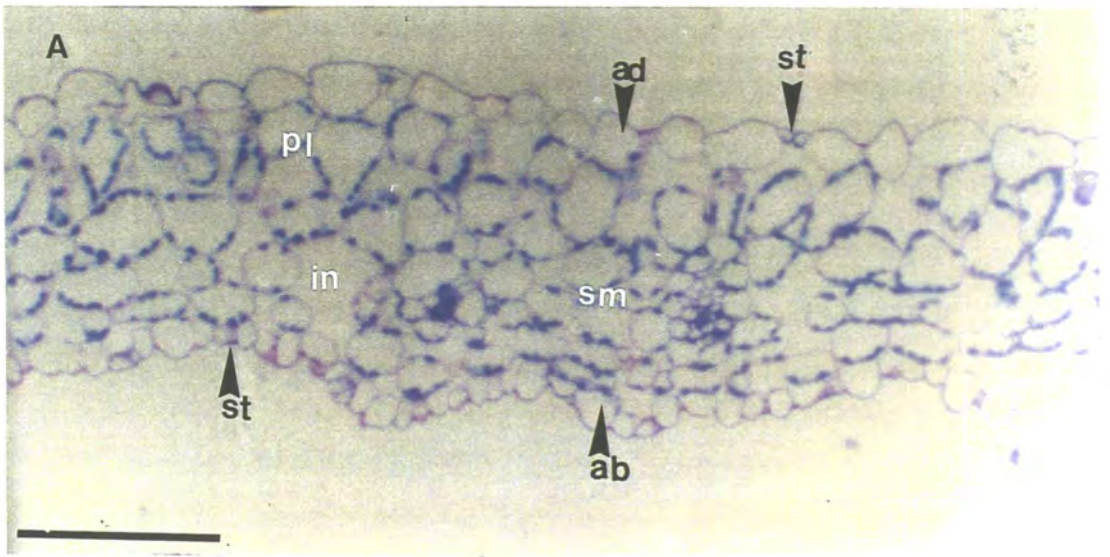


Plate 8.6 A- B. Light micrographs of transverse sections through leaf of *Arabidopsis thaliana* (WT) seedlings, raised under CdSO₄ stress conditions A: control; B: 100 ppm.

Plate 8.6 (B). Notice smaller intercellular spaces and fewer chloroplasts in comparison to the control.

ad, adaxial epidermis; ab, abaxial epidermis; pl, palisade parenchyma; sm, spongy mesophyll; in, intercellular spaces; st, stomata. (Scale bar = 50 μm).

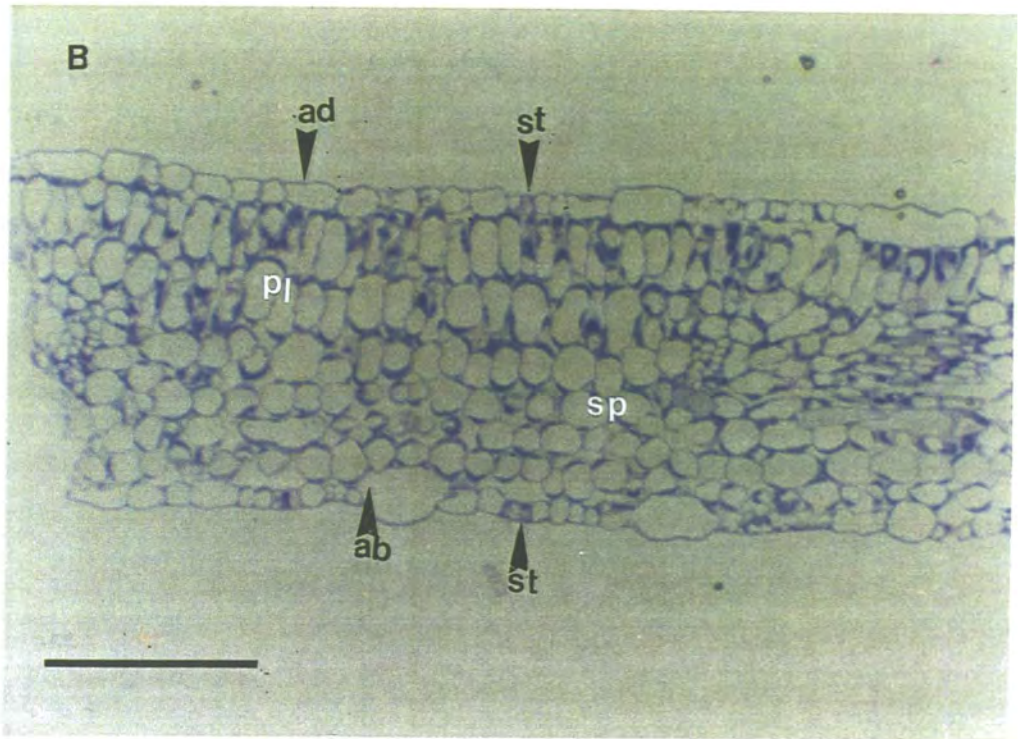
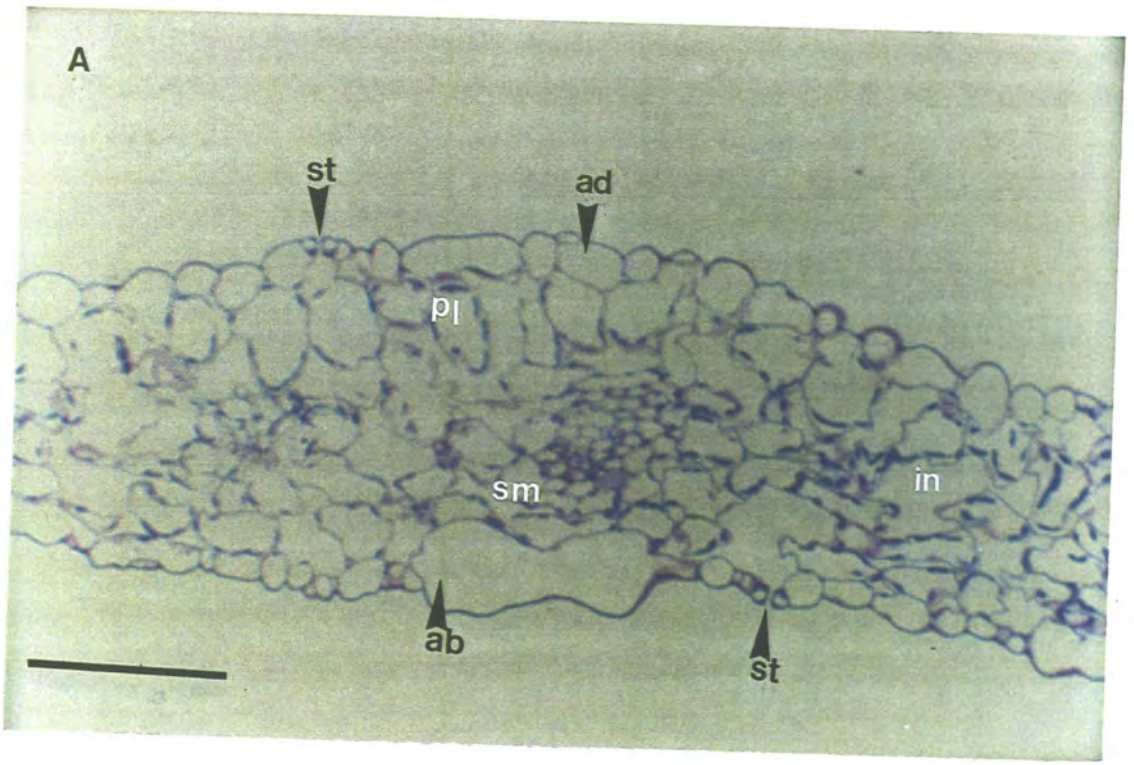


Plate 8.7 A- B. Light micrographs of transverse sections through leaf of *Arabidopsis thaliana* (WT) seedlings, raised under CdSO_4 -stress conditions **A:** 150 ppm; **B:** 200 ppm.

Plate 8.7 (A). Notice loss of chloroplasts in palisade parenchyma and spongy mesophyll.

Plate 8.7 (B). Notice smaller epidermal cells, compressed spongy mesophyll with very small intercellular spaces and loss of chloroplasts.

ad, adaxial epidermis; ab, abaxial epidermis; pl, palisade parenchyma; sm, spongy mesophyll; in, intercellular spaces; st, stomata. (Scale bars = 50 μm).

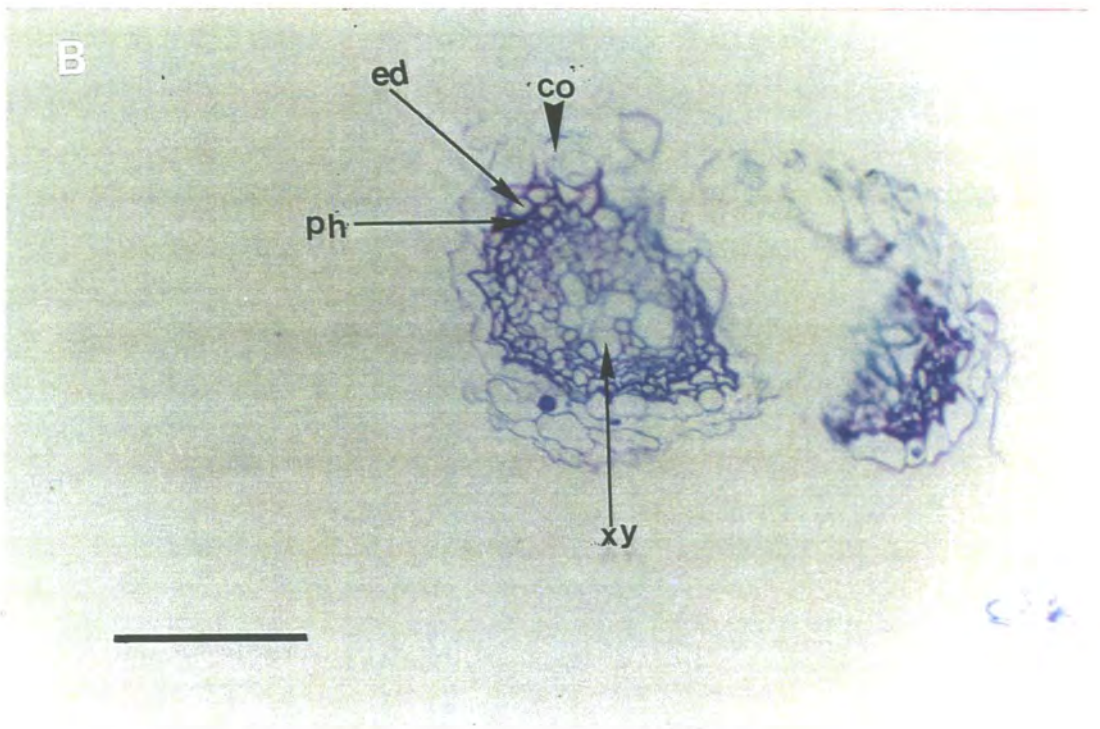
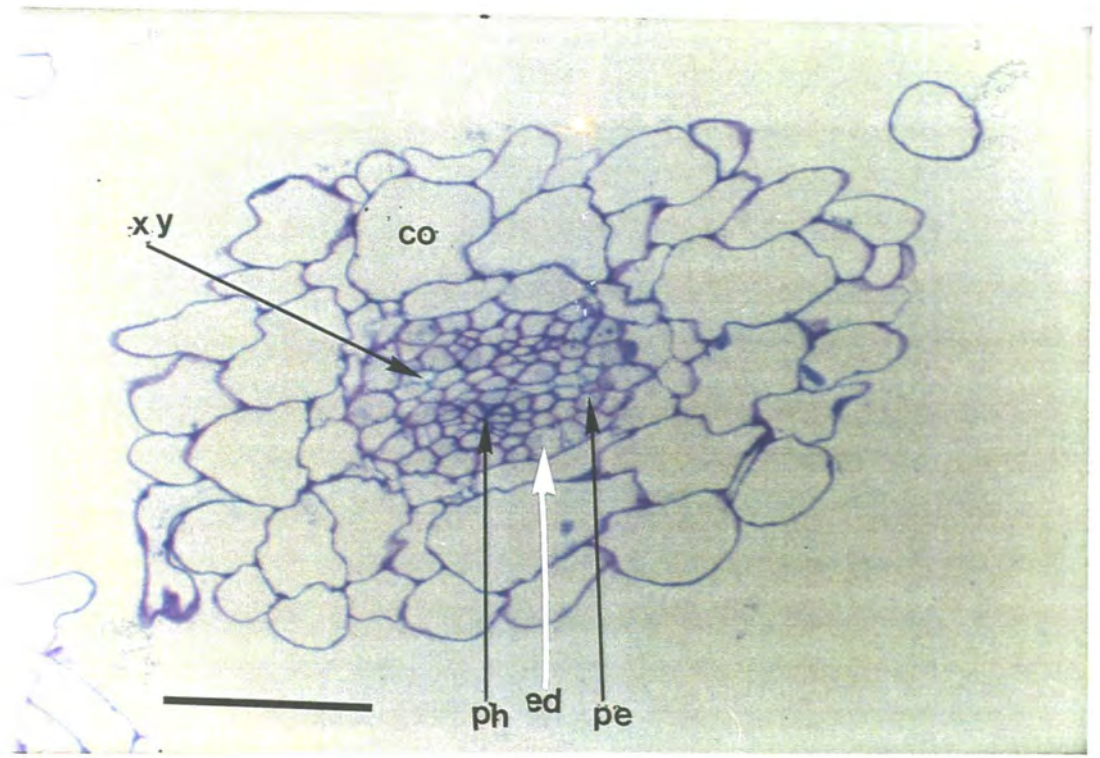


Plate 8.8 A- B. Light micrographs of transverse sections through root of *Arabidopsis thaliana* (WT) seedlings, raised under CdSO₄-stress conditions **A**: control; **B**: 100 ppm.

Plate 8.8 (B). Notice disrupted cortical layers, undifferentiated, highly lignified pericycle and phloem & larger xylem cells.

co, cortex; pe, pericycle; ed, endodermis; ph, phloem; xy, xylem. (Scale bars = 50 μm.).

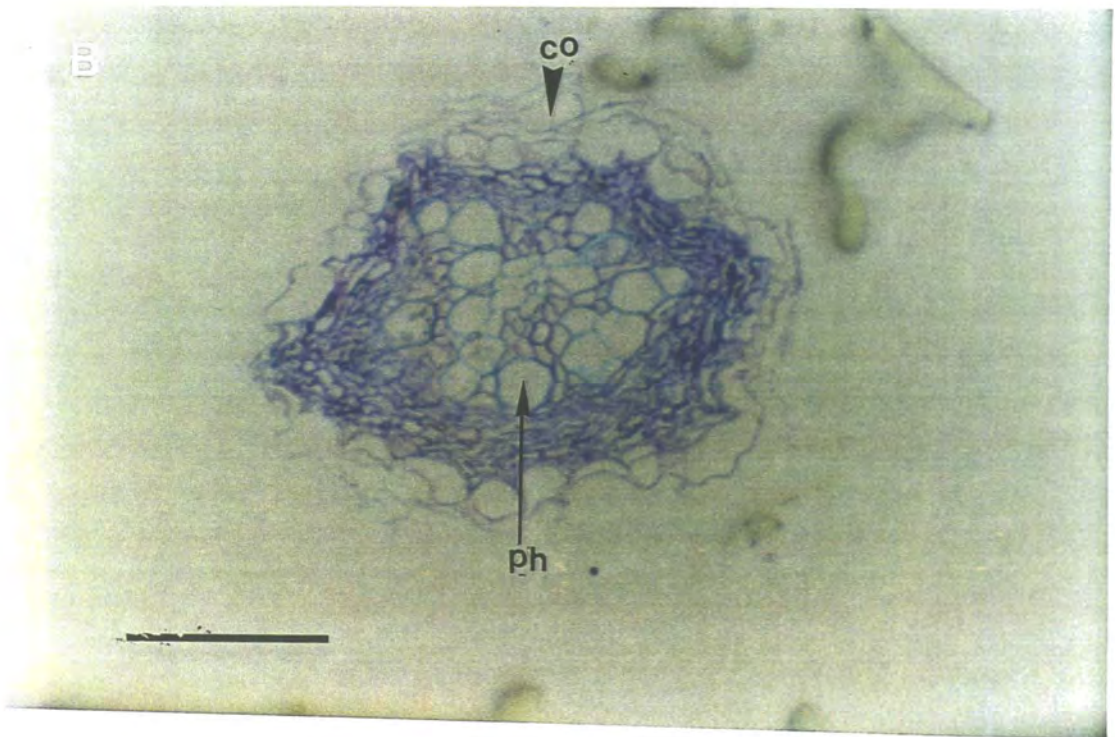
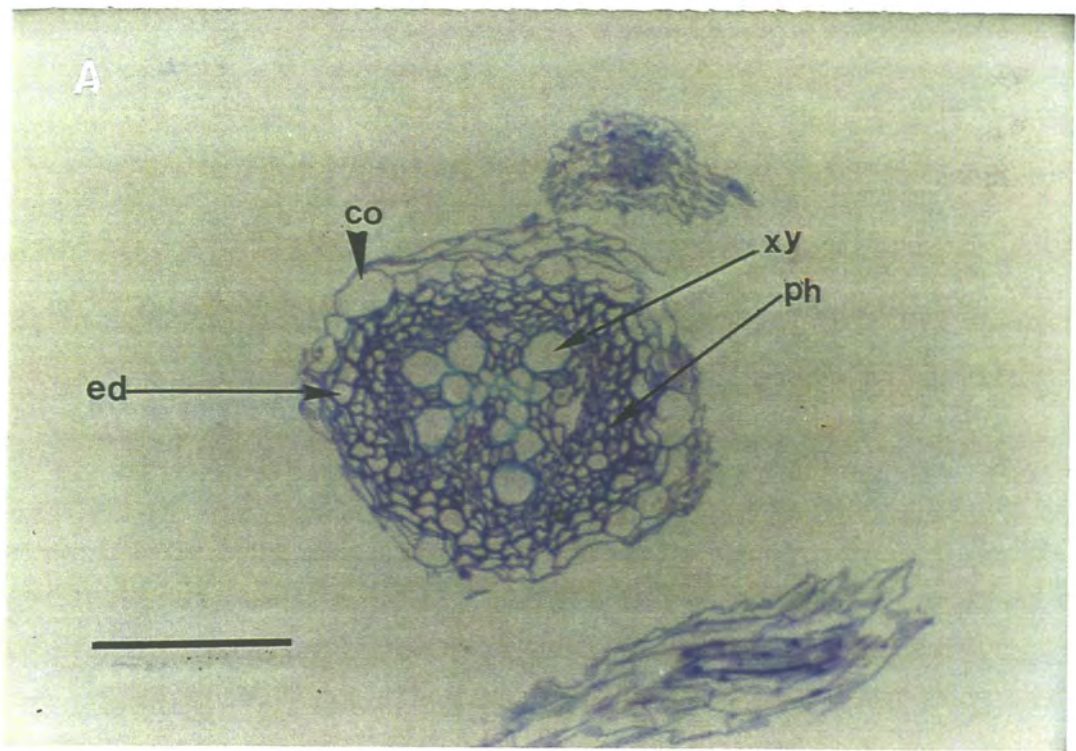


Plate 8.9 A- B. Light micrographs of transverse sections through root of *Arabidopsis thaliana* (WT) seedlings, raised under CdSO_4 -stress conditions **A:** 150 ppm; **B:**200 ppm.

Plate 8.9 (A). Notice disrupted cortical region, damaged endodermis, undifferentiated and highly lignified pericycle, phloem & larger xylem cells.

Plate 8.9 (B). Notice disrupted cortical region and endodermis, highly lignified and undistinguishable pericycle, phloem, & larger xylem cells.

co, cortex; ed, endodermis; ph, phloem; xy, xylem. (Scale bars = 50 μm .)

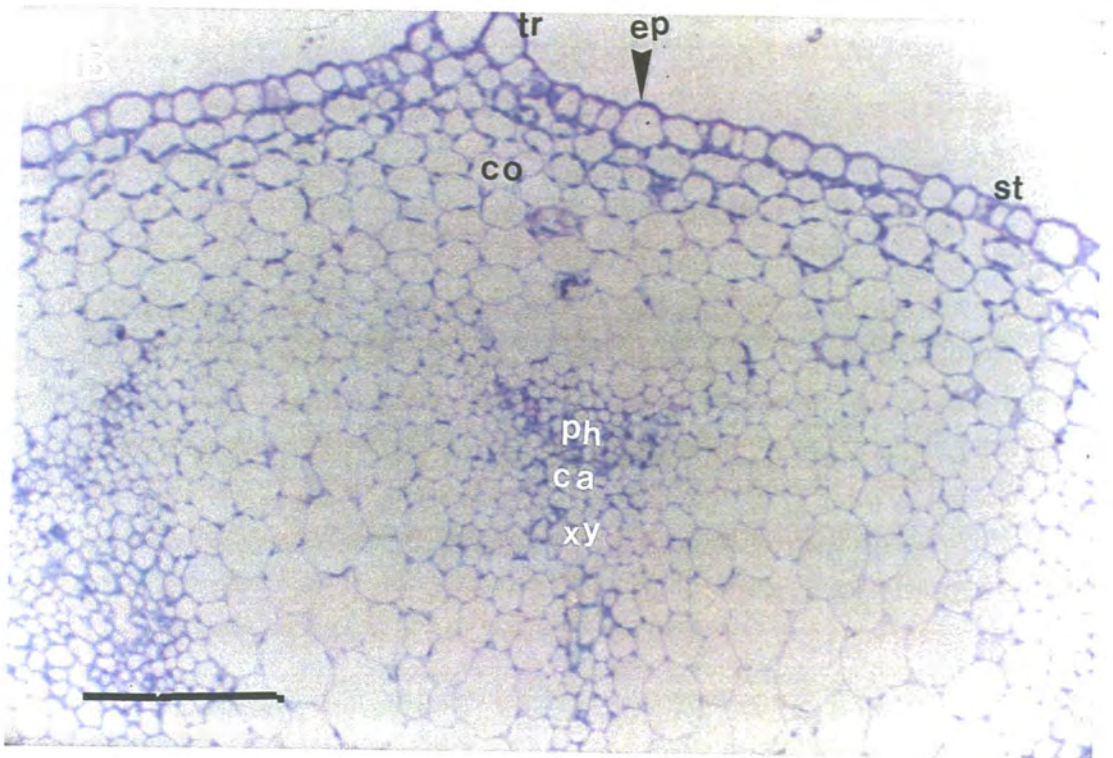
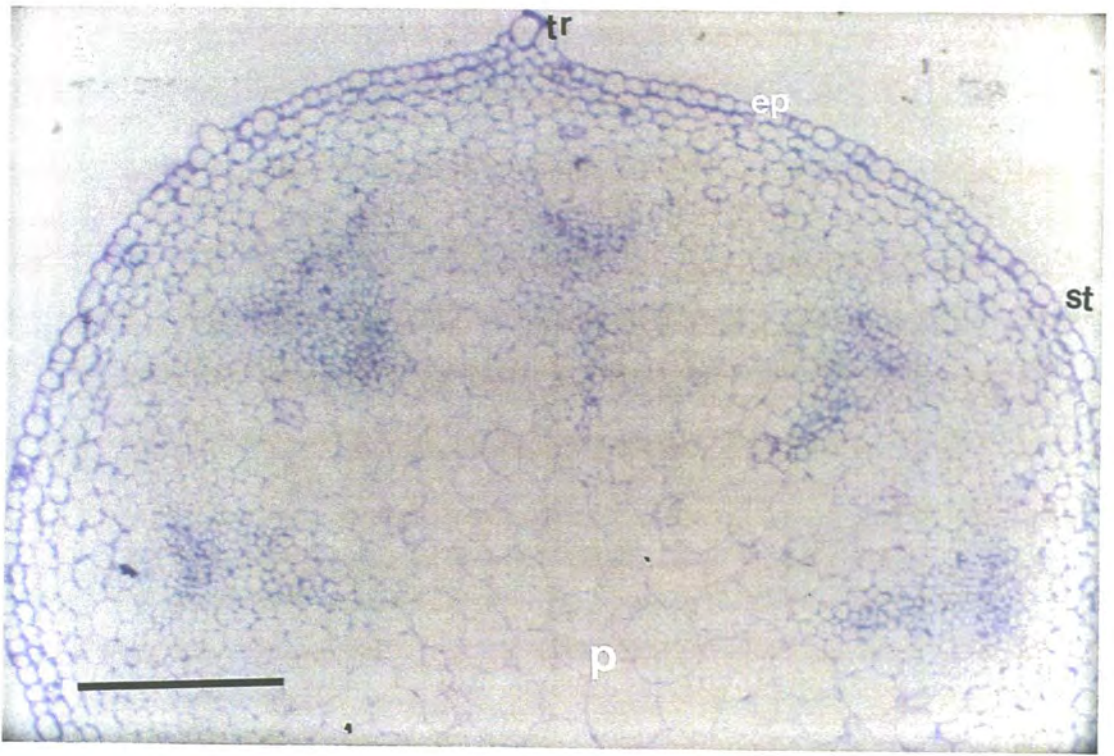


Plate 8.10 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (CAD1) seedlings, raised without CdSO₄-stress conditions (control). tr, trichome; ep, epidermis; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μ m, B = 50 μ m).

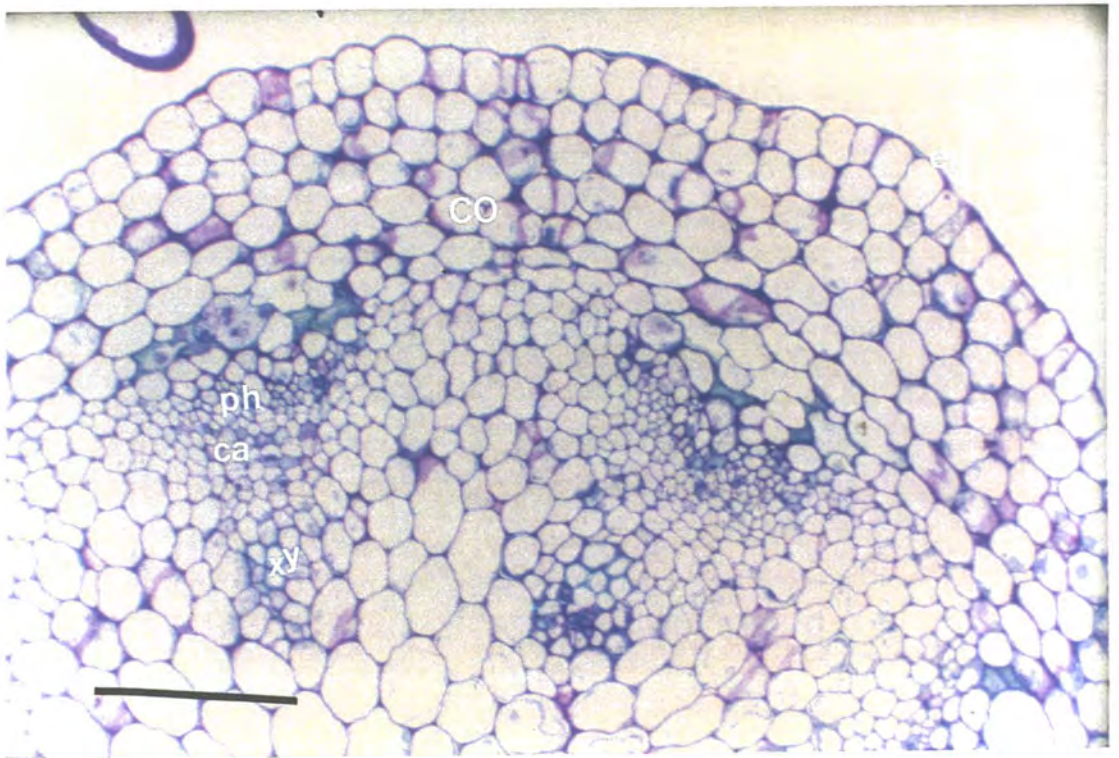
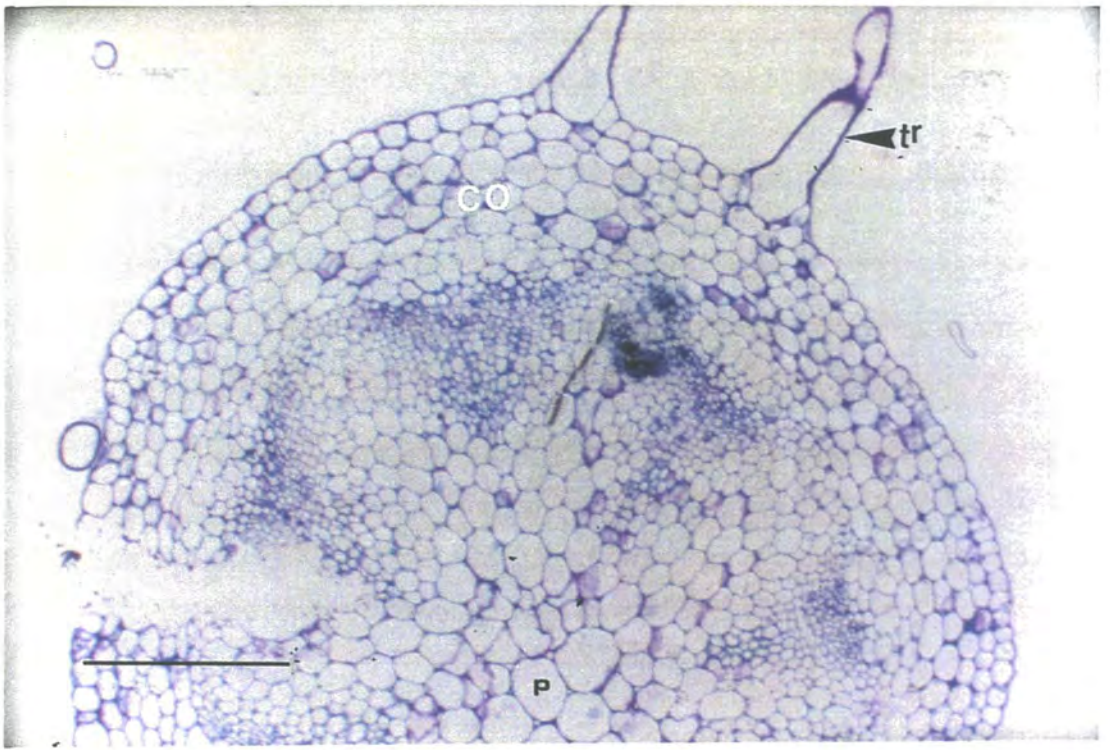


Plate 8.11 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (CAD1) seedlings, raised under CdSO₄-stress conditions (100 ppm).

Plate 8.11 (A). Notice slightly irregular stem shape.

Plate 8.11 (B). Notice larger epidermal cells & smaller pith cells.

ep, epidermis; st, stomata; co, cortex; pc, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm, B = 50 μm)

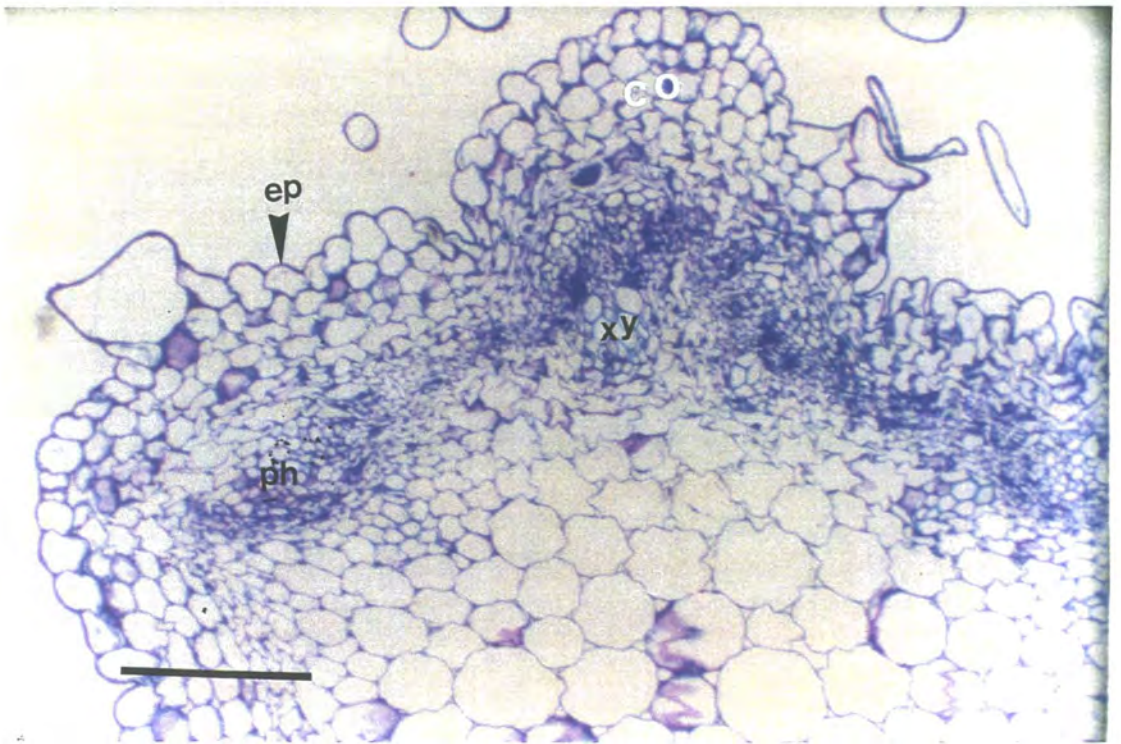
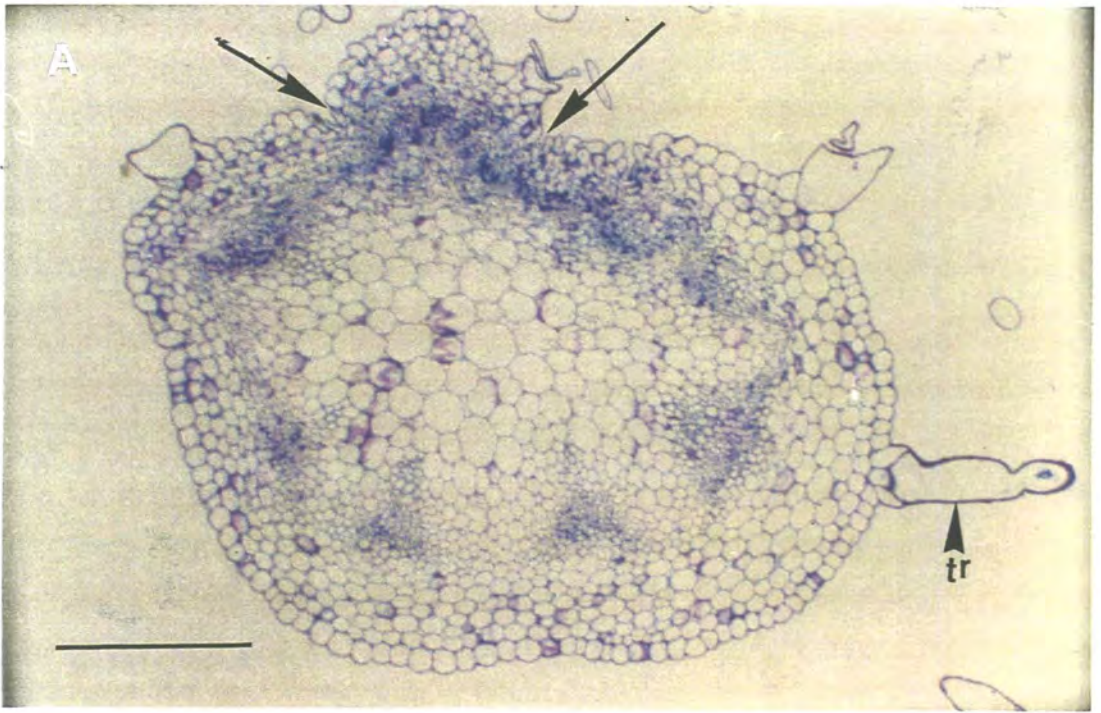


Plate 8.12 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (CAD1) seedlings, raised under CdSO₄-stress conditions (150 ppm). **Plate 8.12 (A).** Notice striking change in the stem shape and disrupted (arrowed) regions.

Plate 8.12 (B). Notice the undifferentiated cortical parenchyma cells and vascular bundles in the disrupted stem region.

tr, trichome; ep, epidermis; st, stomata; co, cortex; pc, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm, B = 50 μm).

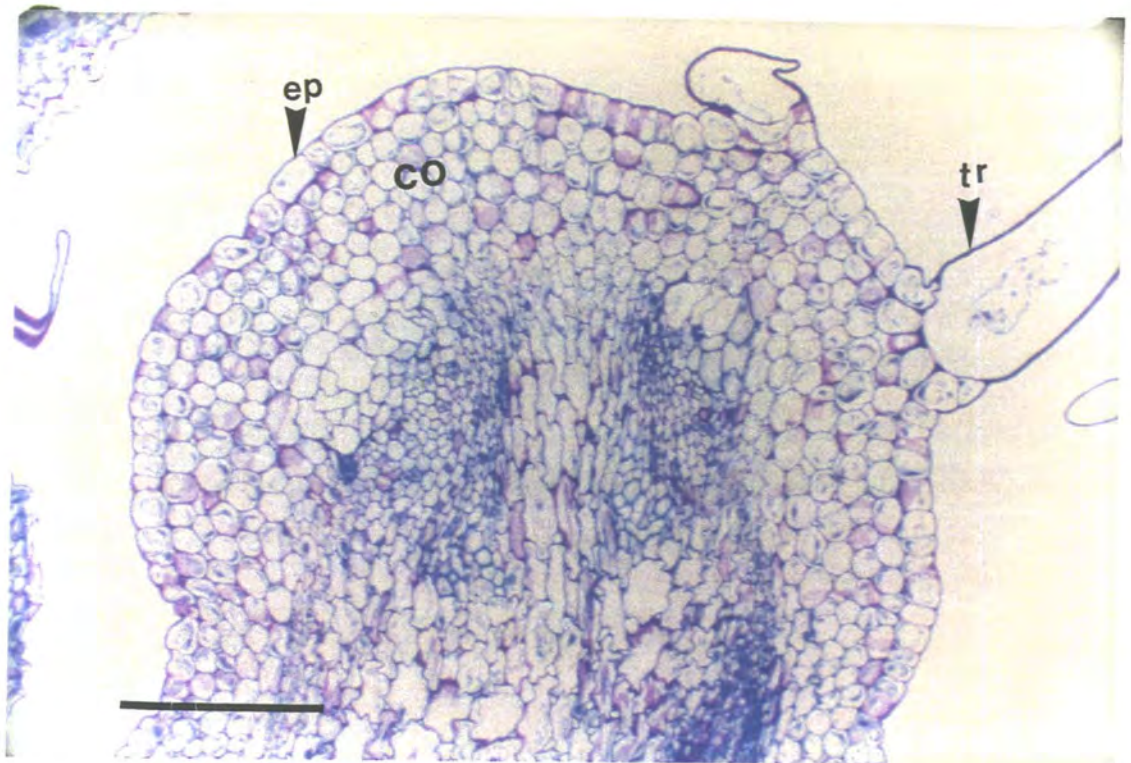
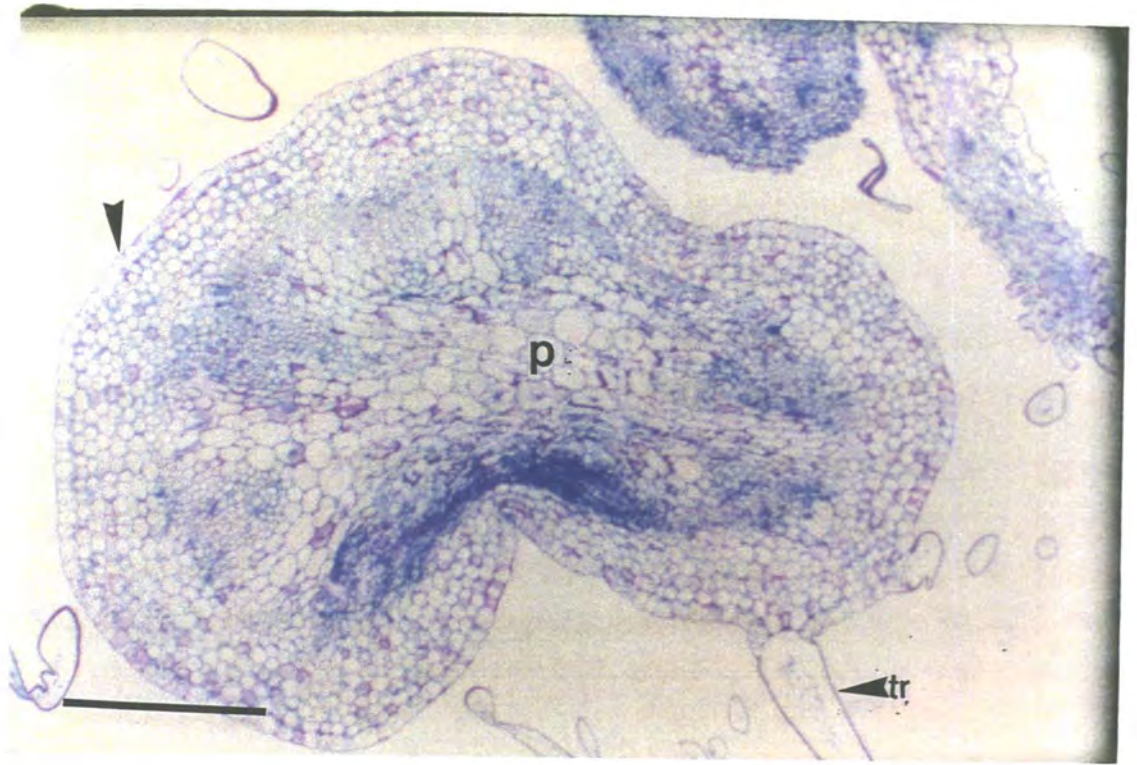


Plate 8.13 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (CAD1) seedlings, raised under CdSO₄-stress conditions (200 ppm).

Plate 8.13 (A). Notice striking change in the stem shape.

Plate 8.13 (B). Notice the undifferentiation of phloem, cambium and xylem in the disrupted region of stem.

tr, trichome; ep, epidermis; st, stomata; co, cortex; pc, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm, B = 50 μm).

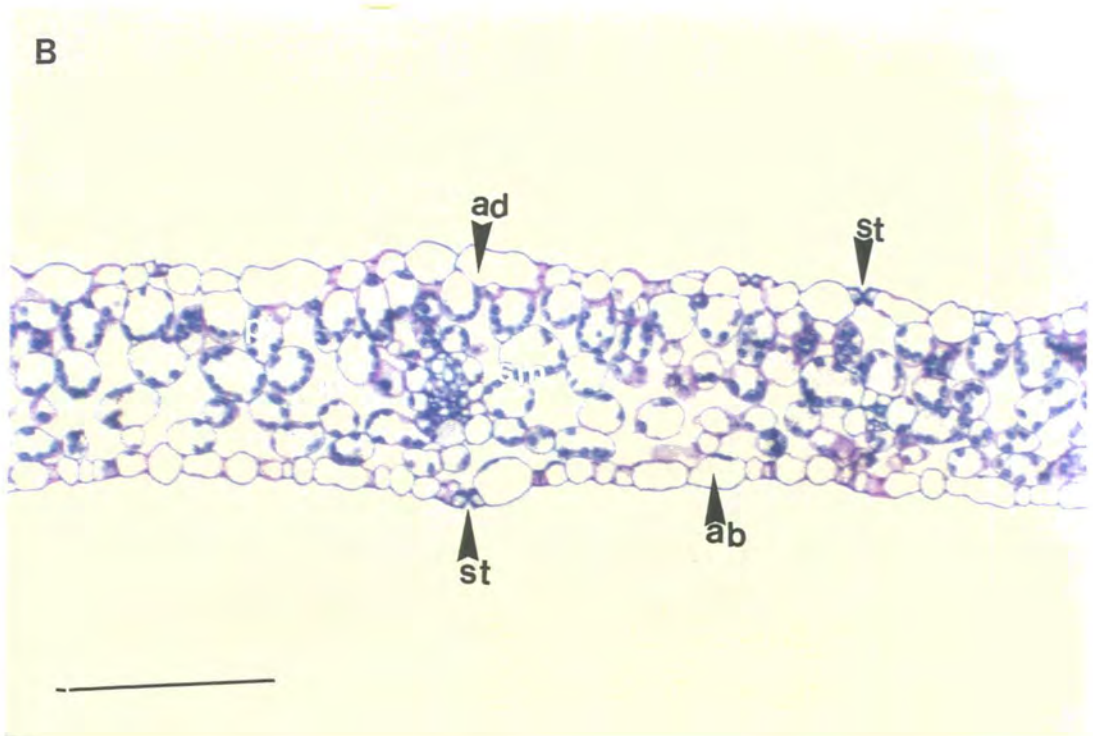
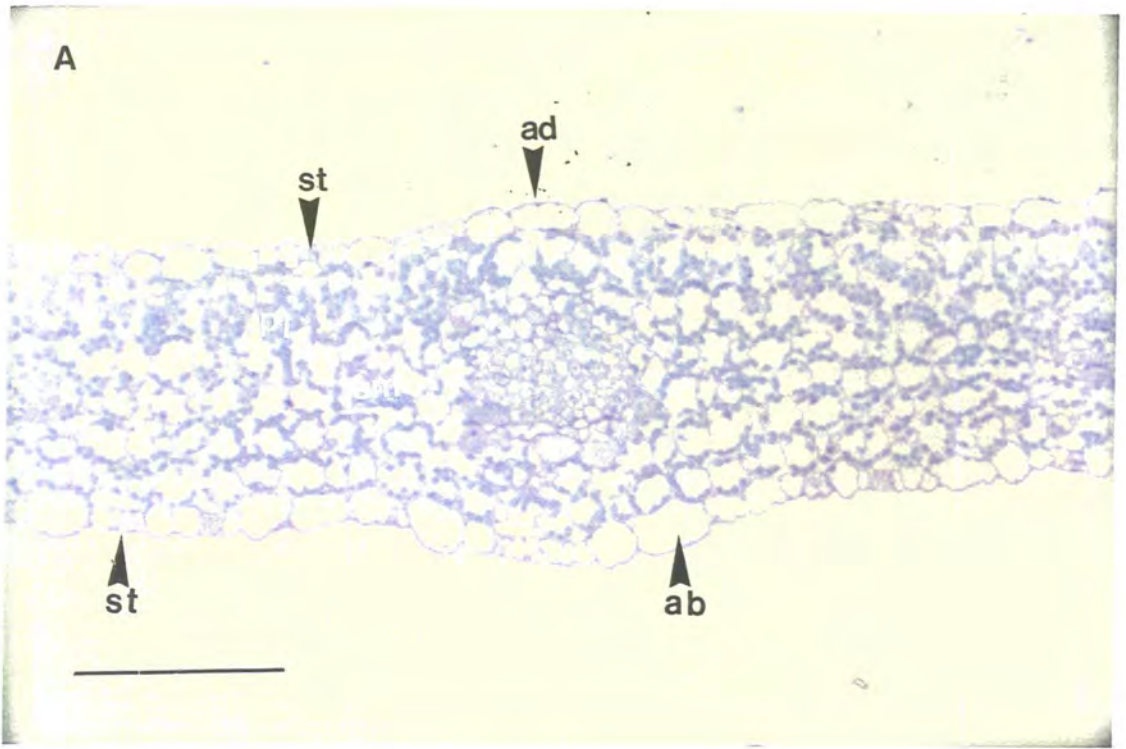


Plate 8.14 A- B. Light micrographs of transverse sections through leaf of *Arabidopsis thaliana* (*CAD1*) seedlings, raised under CdSO_4 stress conditions **A**: control; **B**: 100 ppm.

Plate 8.14 (B). Notice larger intercellular spaces in spongy mesophyll and lower chloroplasts than control.

ad, adaxial epidermis; ab, abaxial epidermis; pl, palisade parenchyma; sm, spongy mesophyll; in, intercellular spaces; st, stomata. (Scale bars = 50 μm).

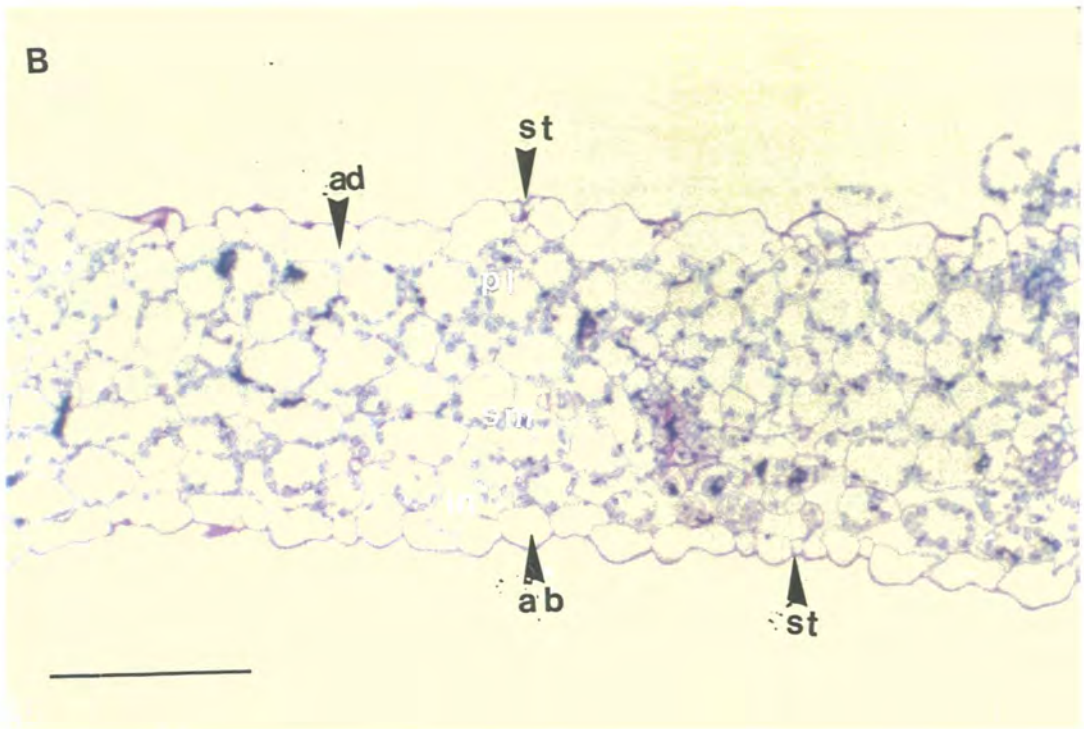
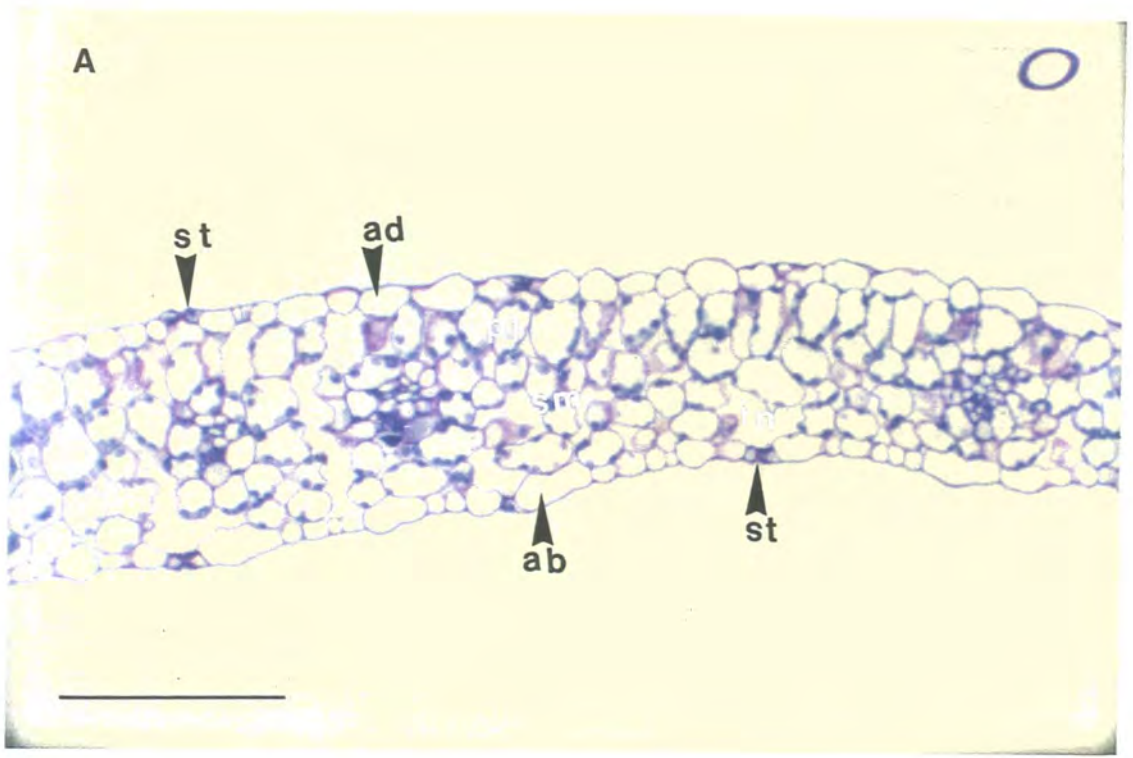


Plate 8.15 A- B. Light micrographs of transverse sections through leaf of *Arabidopsis thaliana* (CAD1) seedlings, raised under CdSO_4 stress conditions A: 150 ppm; B: 200 ppm. ad, adaxial epidermis; ab, abaxial epidermis; pl, palisade parenchyma; sm, spongy mesophyll; in, intercellular spaces; st, stomata. (Scale bars = 50 μm).

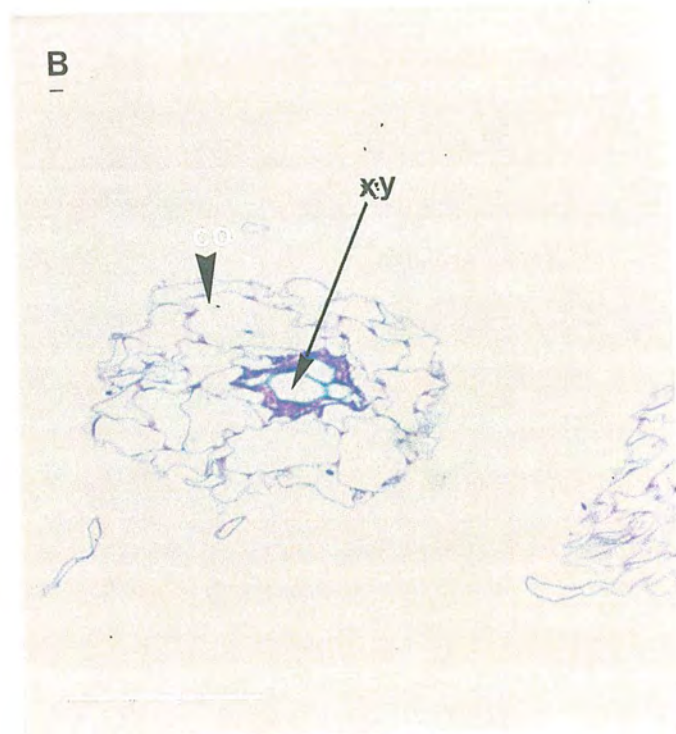
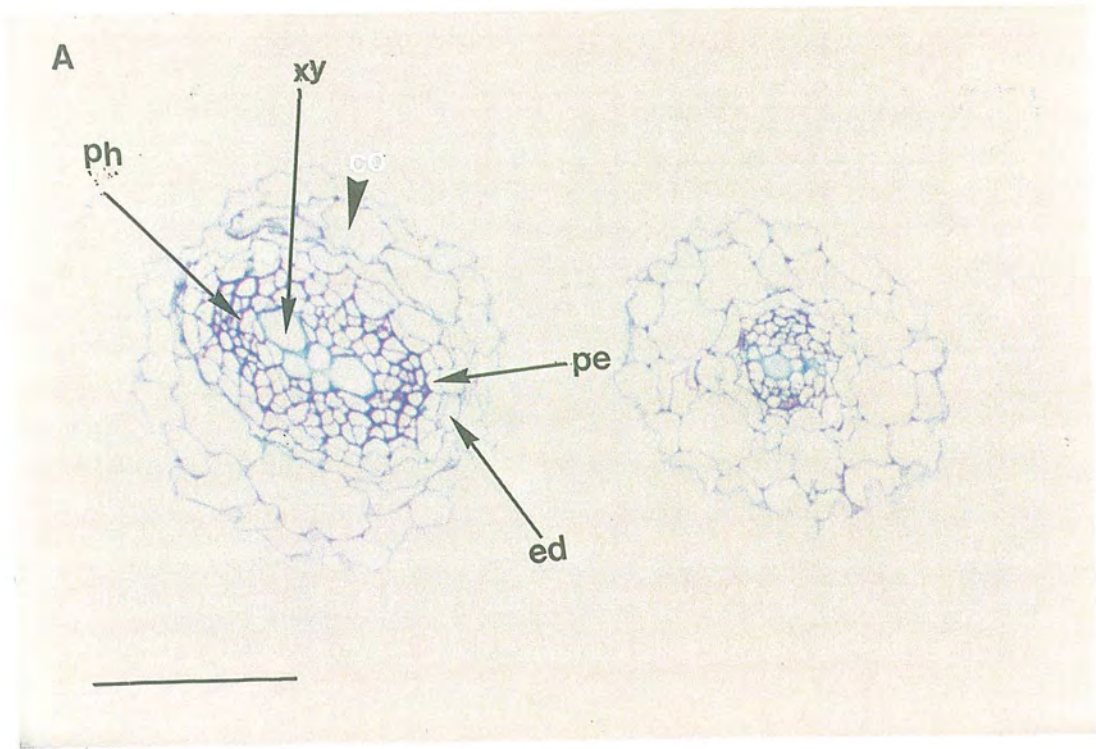


Plate 8.16 A- B. Light micrographs of transverse sections through root of *Arabidopsis thaliana* (CAD1) seedlings, raised under CdSO₄-stress conditions. A: control; B: 100 ppm. co, cortex; pe, pericycle; ed, endodermis; ph, phloem; xy, xylem. (Scale bars = 50 μm.).

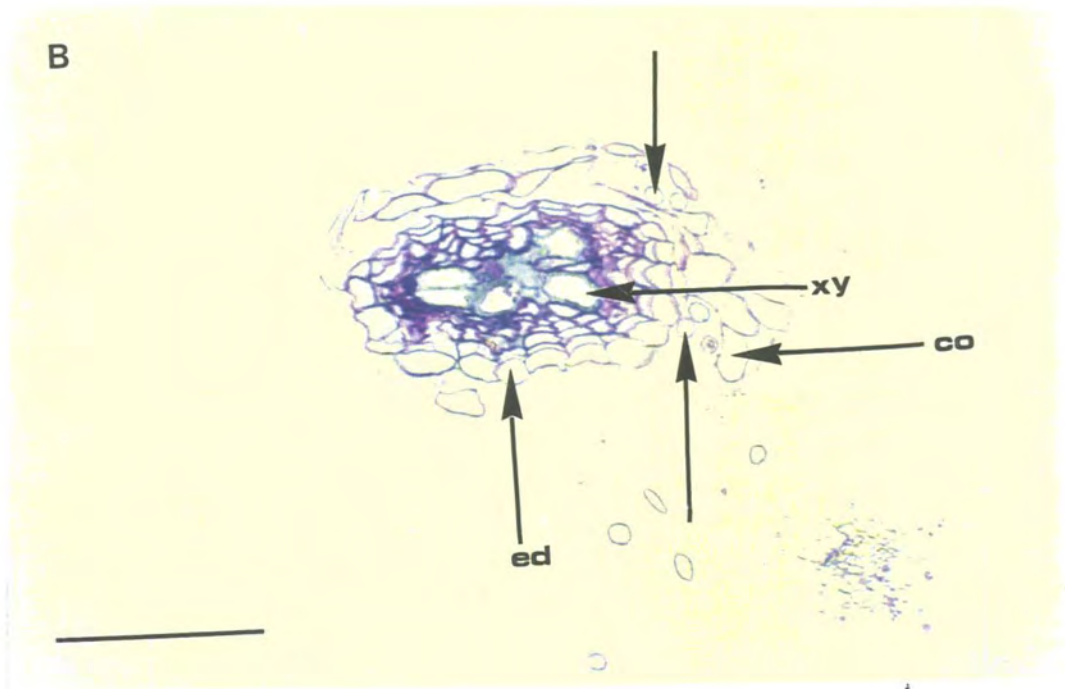
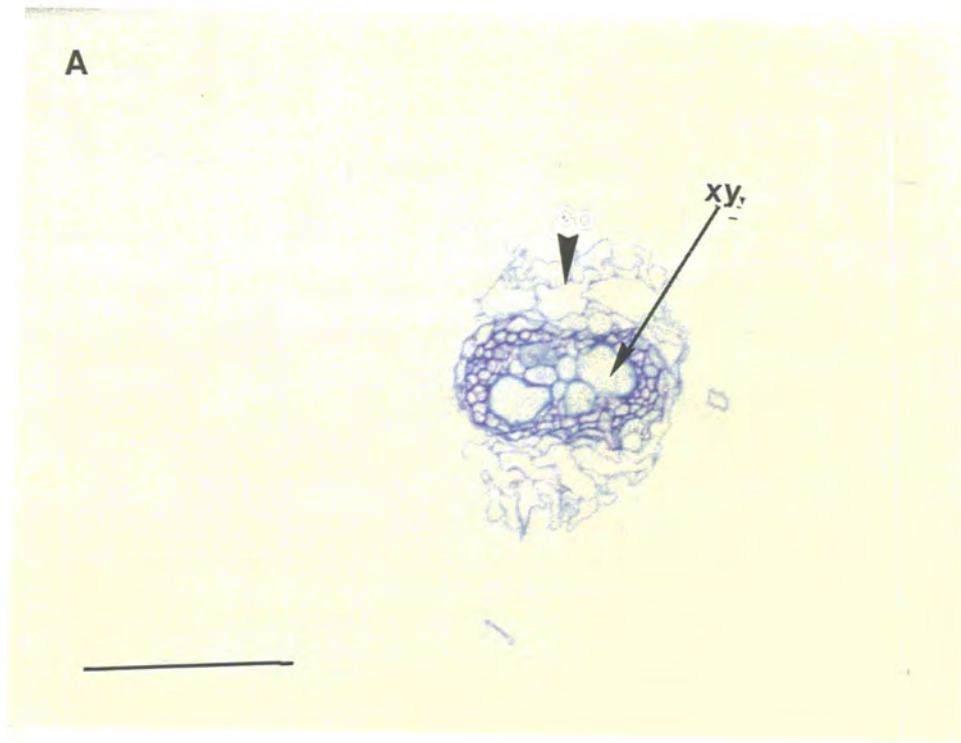


Plate 8.17 A- B. Light micrographs of transverse sections through root of *Arabidopsis thaliana* (CAD1) seedlings, raised under CdSO_4 -stress conditions **A:** 150; **B:**200 ppm.

Plate 17. (A). Notice some unusual cells (arrowed).
 co, cortex; ed, endodermis; ph, phloem; xy, xylem, (Scale bars = 50 μm .)

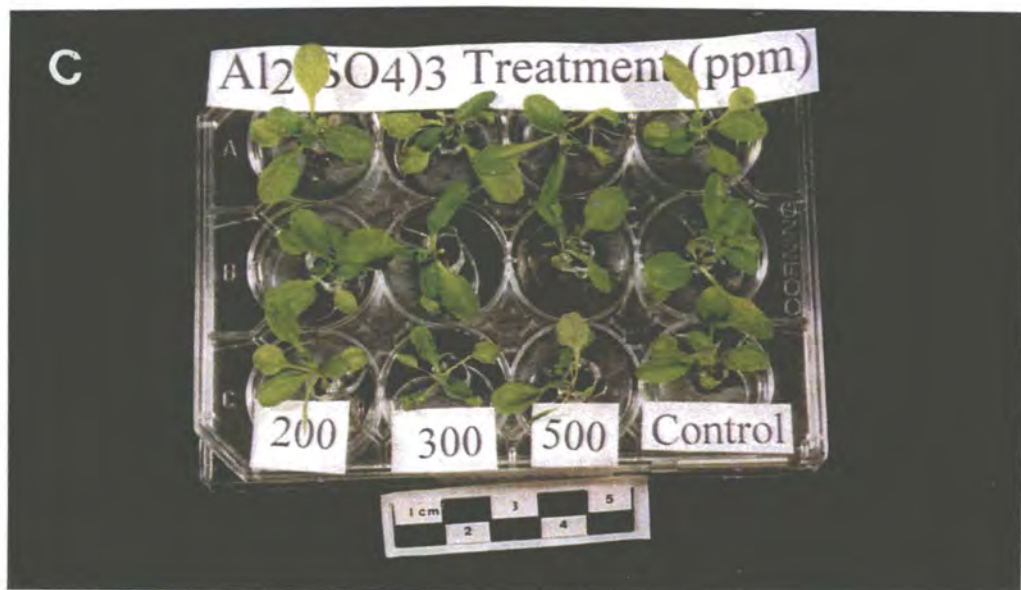
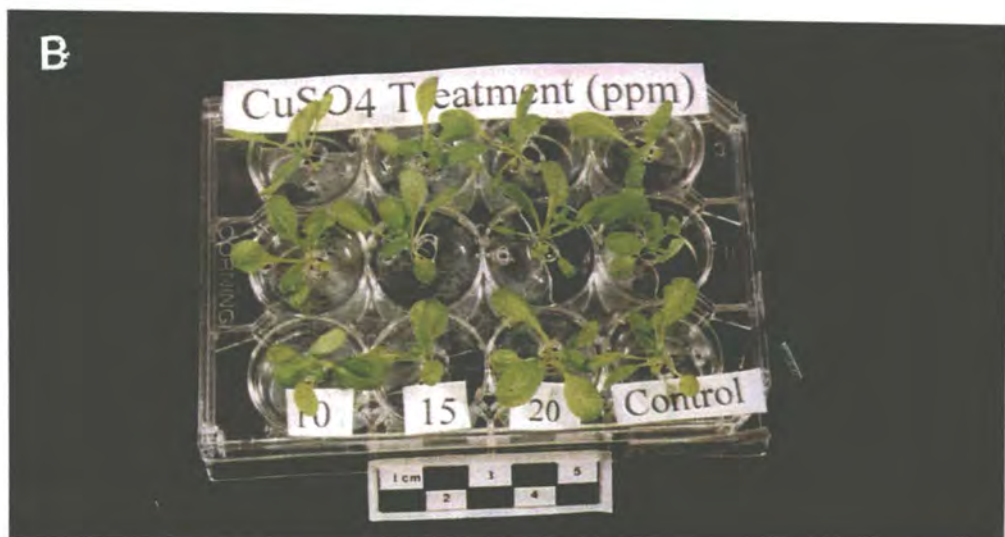
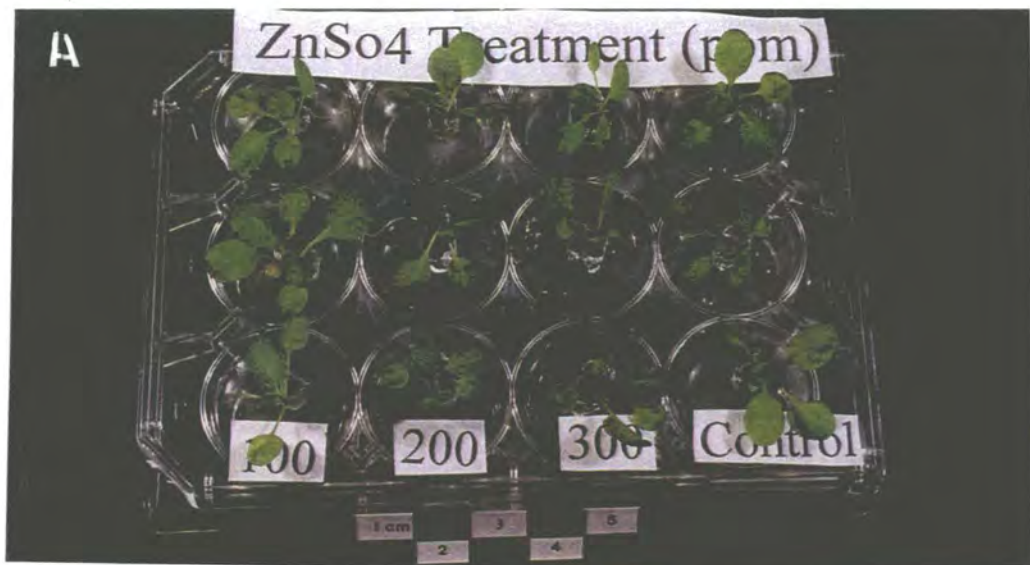


Plate 8.18 A-C. Seedlings of *Arabidopsis thaliana* grown under heavy metal stress regimes , A, ZnSO₄ ; B, CuSO₄ & C, Al₂(SO₄)₃. for observing the anatomical changes in the stem, leaf and root.

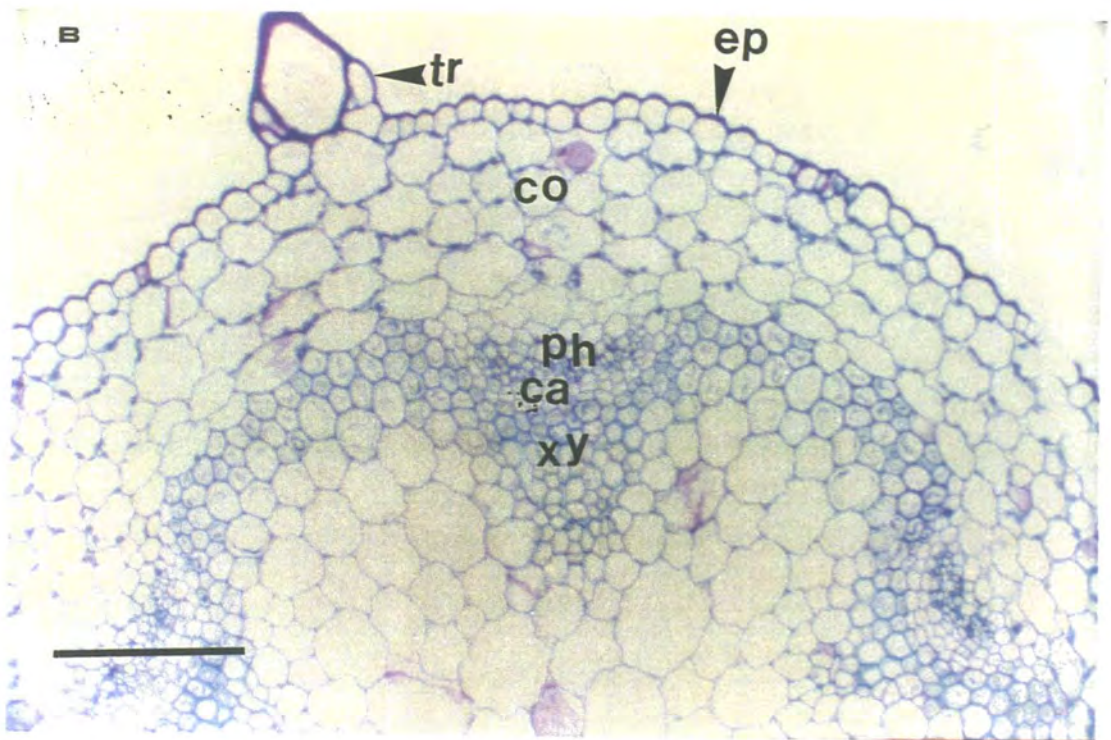
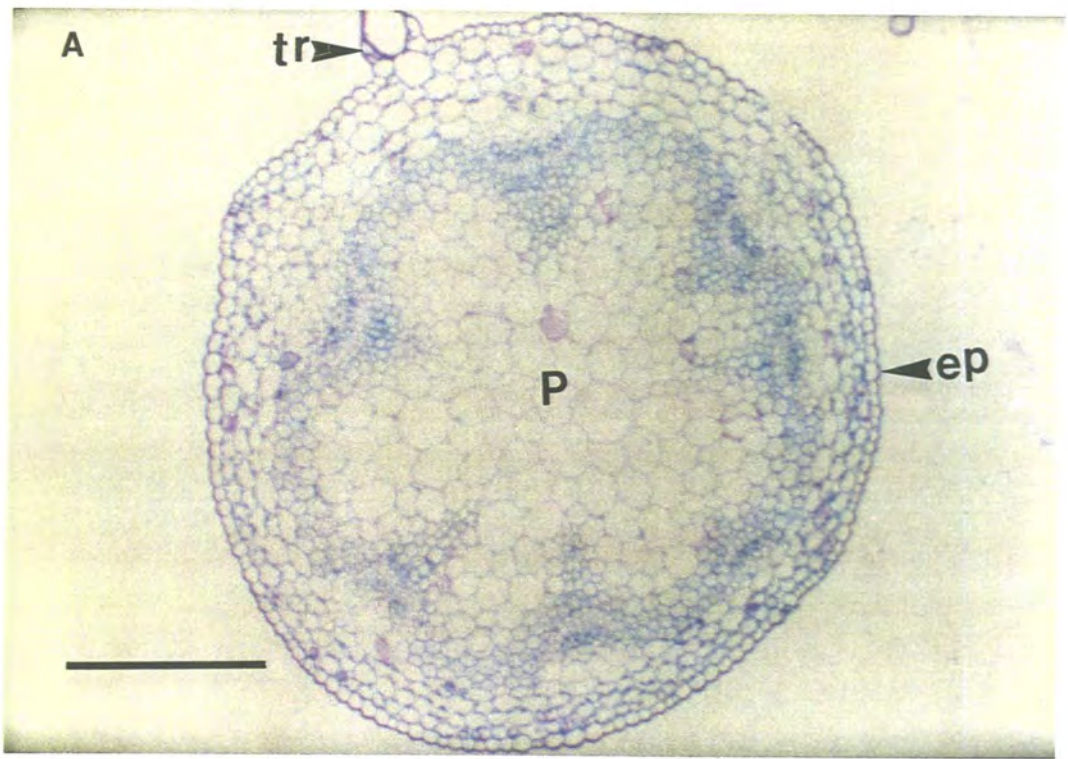


Plate 8.19 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under $\text{Al}_2(\text{SO}_4)_3$ -stress conditions (200 ppm). ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm).

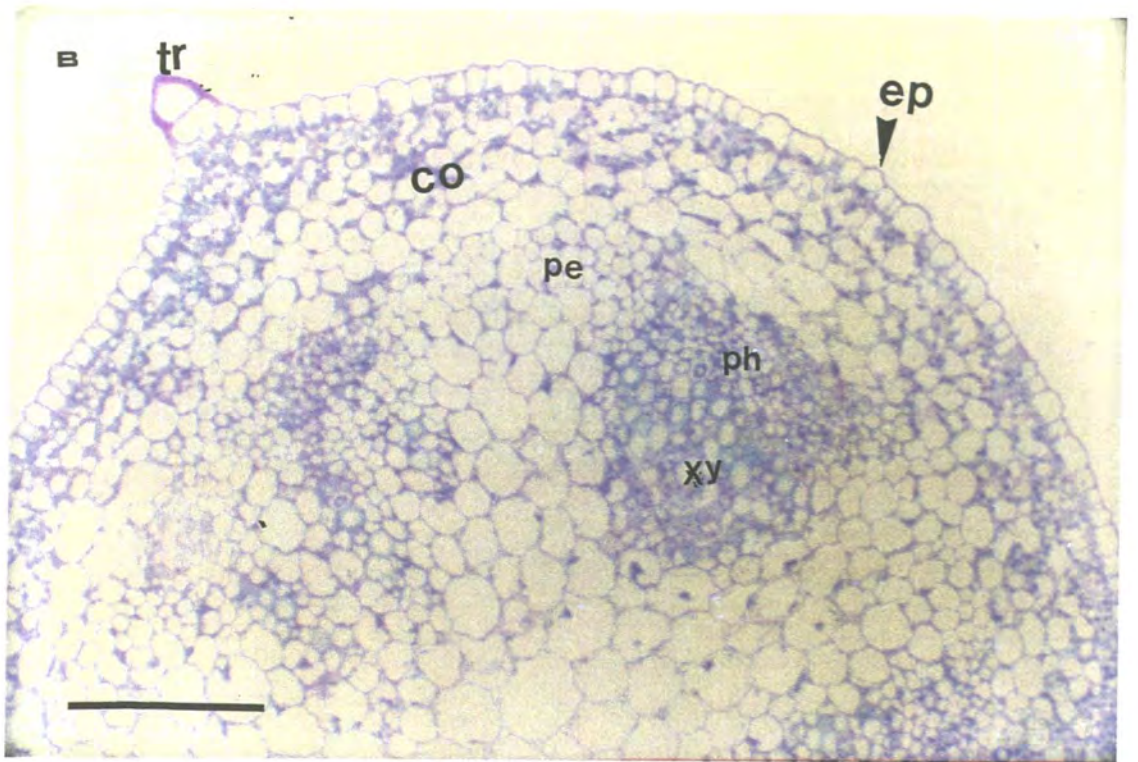
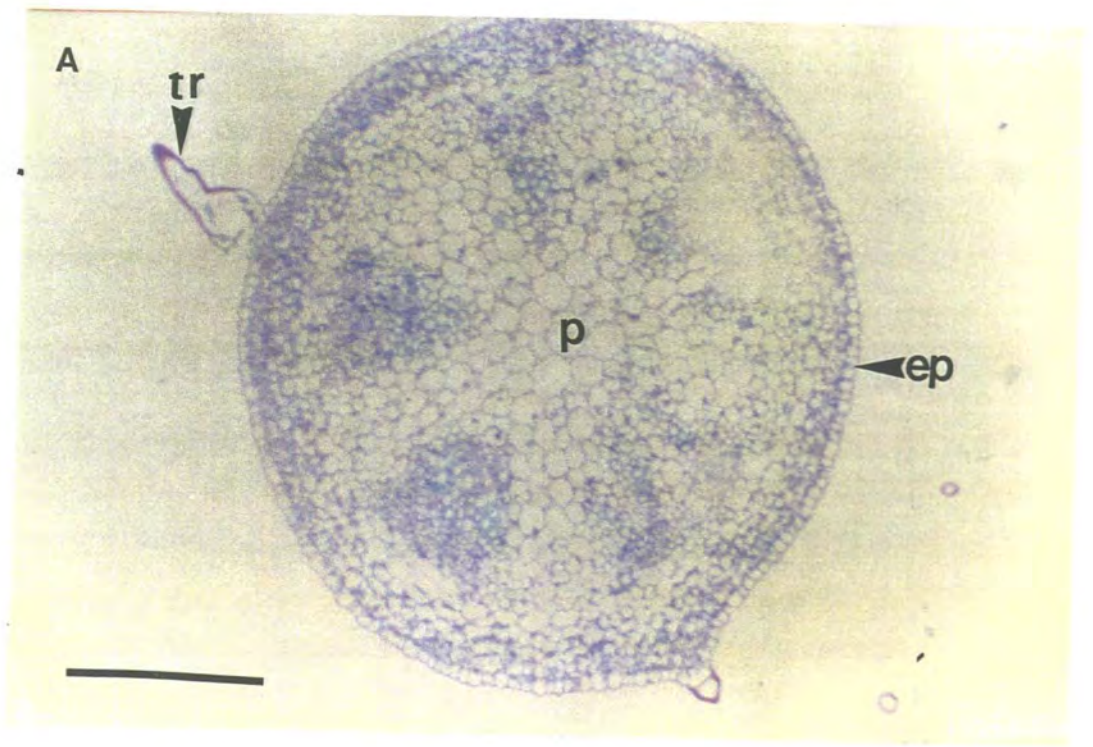


Plate 8.20 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under $\text{Al}_2(\text{SO}_4)_3$ -stress conditions (300 ppm). tr, trichome; ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm).

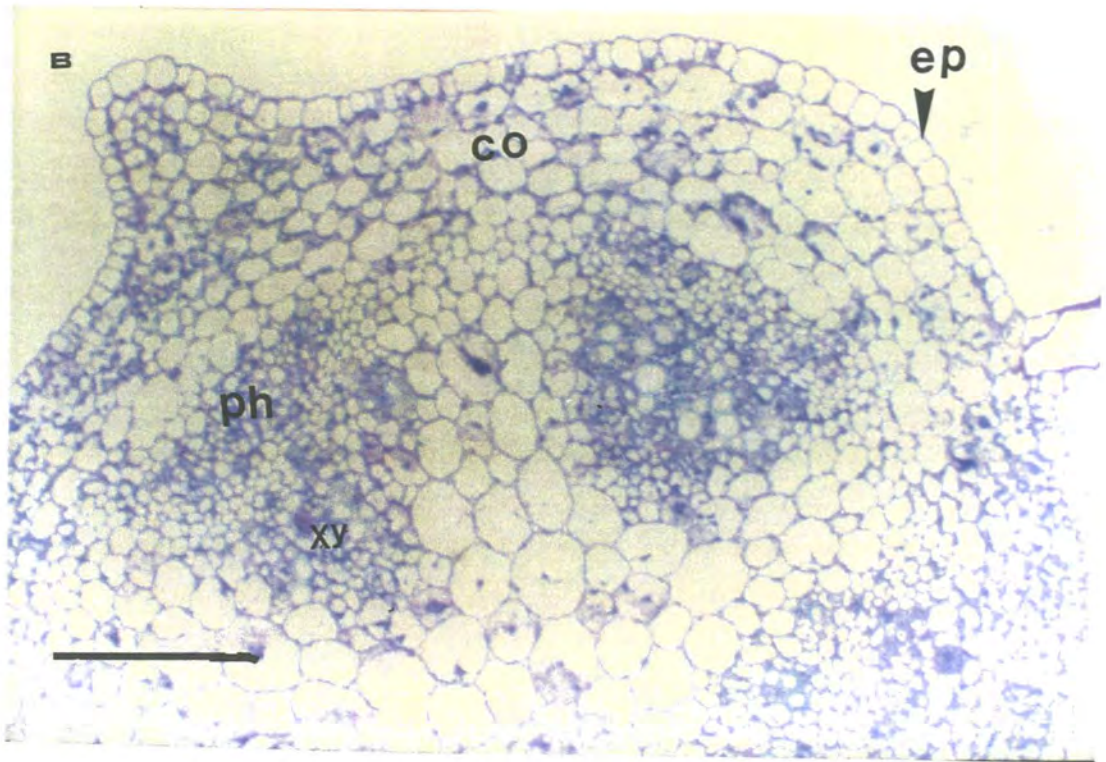
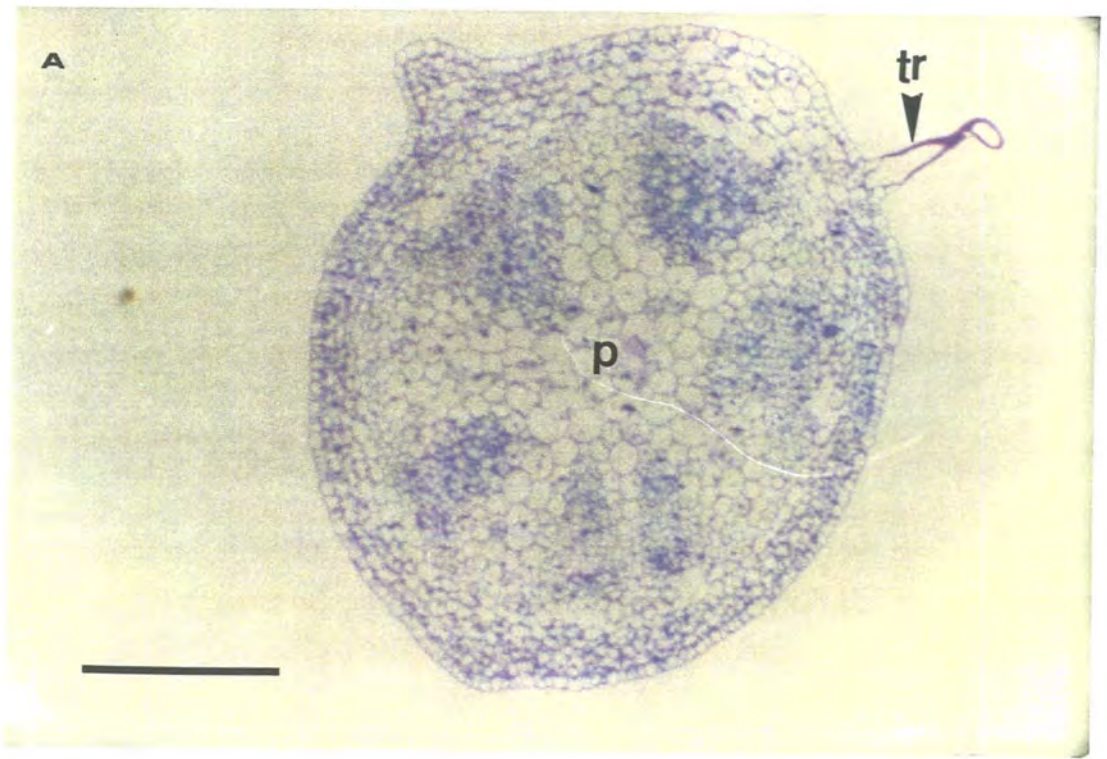


Plate 8.21 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under $\text{Al}_2(\text{SO}_4)_3$ -stress conditions (500 ppm). tr, trichome; ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm).

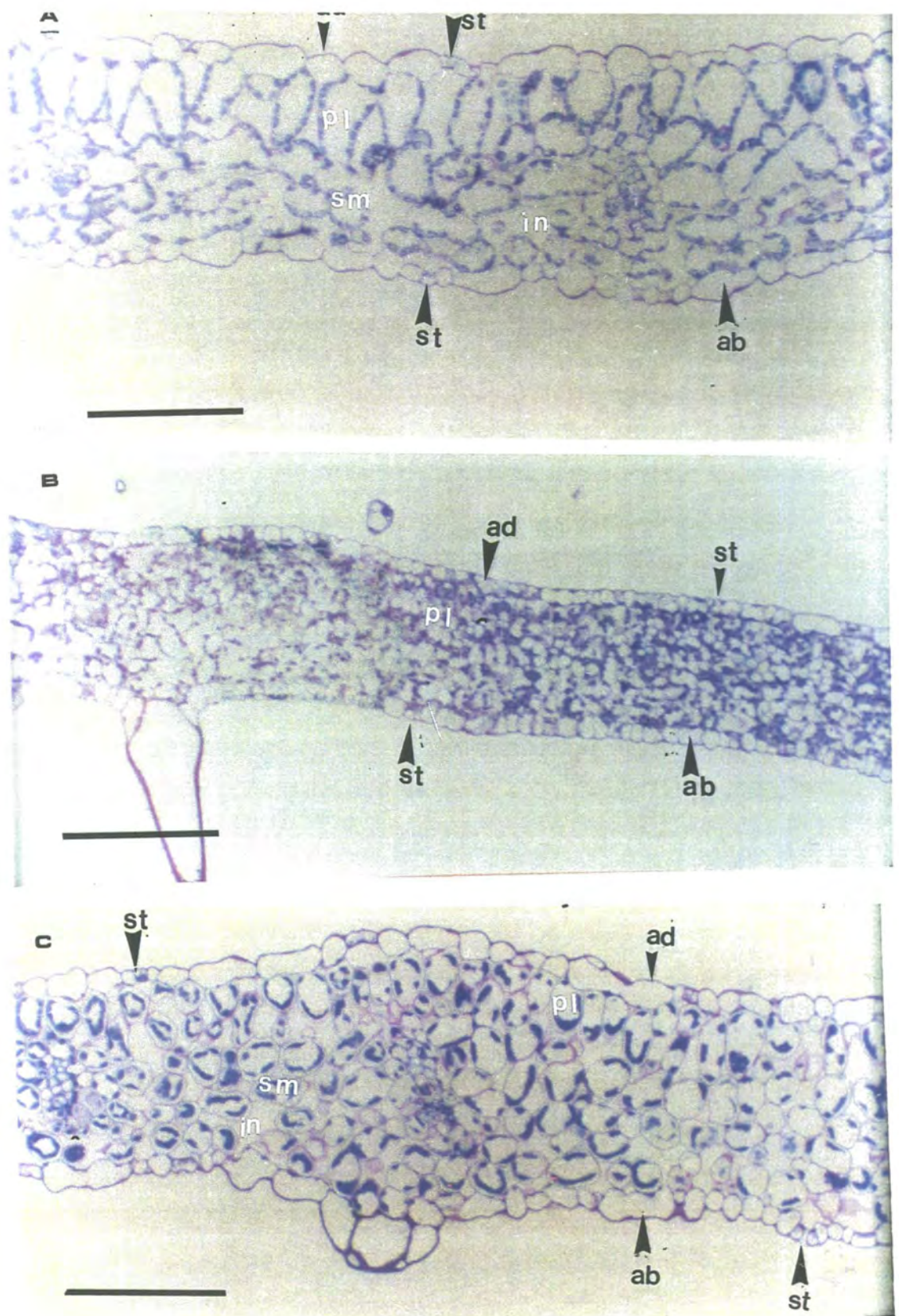


Plate 8.22 A-C. Light micrographs of transverse sections through leaf of *Arabidopsis thaliana* (WT) seedlings, raised under $\text{Al}_2(\text{SO}_4)_3$ stress conditions A: 200 ppm; B: 300 ppm; C: 500 ppm.

Plate 8.22 (A). Notice smaller intercellular spaces in spongy mesophyll and more chloroplasts than control.

Plate 8.22 (B). Notice smaller cells and intercellular spaces in palisade parenchyma and spongy mesophyll & more chloroplasts.

Plate 8.22 (C). Notice smaller palisade parenchyma and spongy mesophyll cells, smaller intercellular spaces & more chloroplasts.

ad, adaxial epidermis; ab, abaxial epidermis; pl, palisade parenchyma; sm, spongy mesophyll; in, intercellular spaces; st, stomata. (Scale bars = 50 μm).

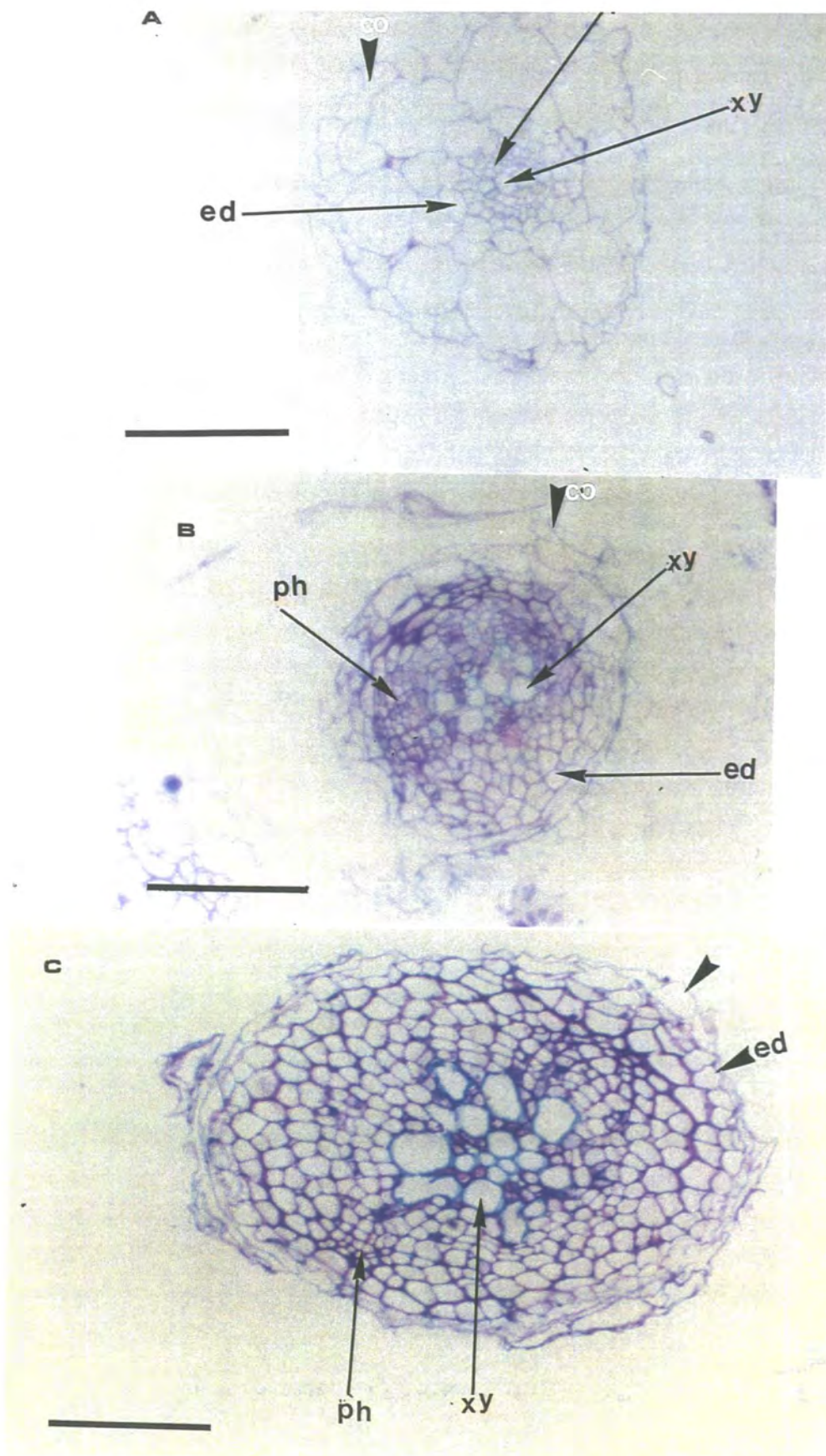


Plate 8.23 A- C Light micrographs of transverse sections through root of *Arabidopsis thaliana* (WT) seedlings, raised under $\text{Al}_2(\text{SO}_4)_3$ -stress conditions A: 200; B:300 ppm; C: 500 ppm.

Plate 8.23 (B). Notice disrupted cortical region and endodermis.

Plate 8.23 (C). Notice disrupted cortical region, endodermis & large xylem cells.

co, cortex; ed, endodermis; ph, phloem; xy, xylem. (Scale bars = 50 μm .)

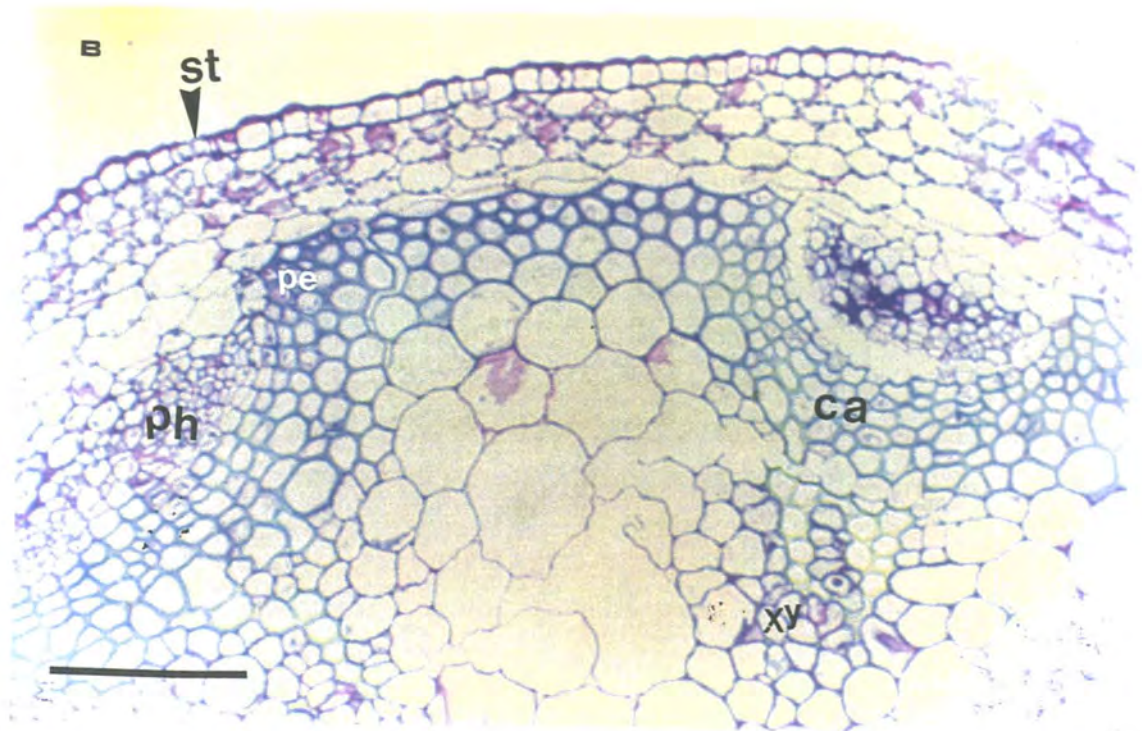
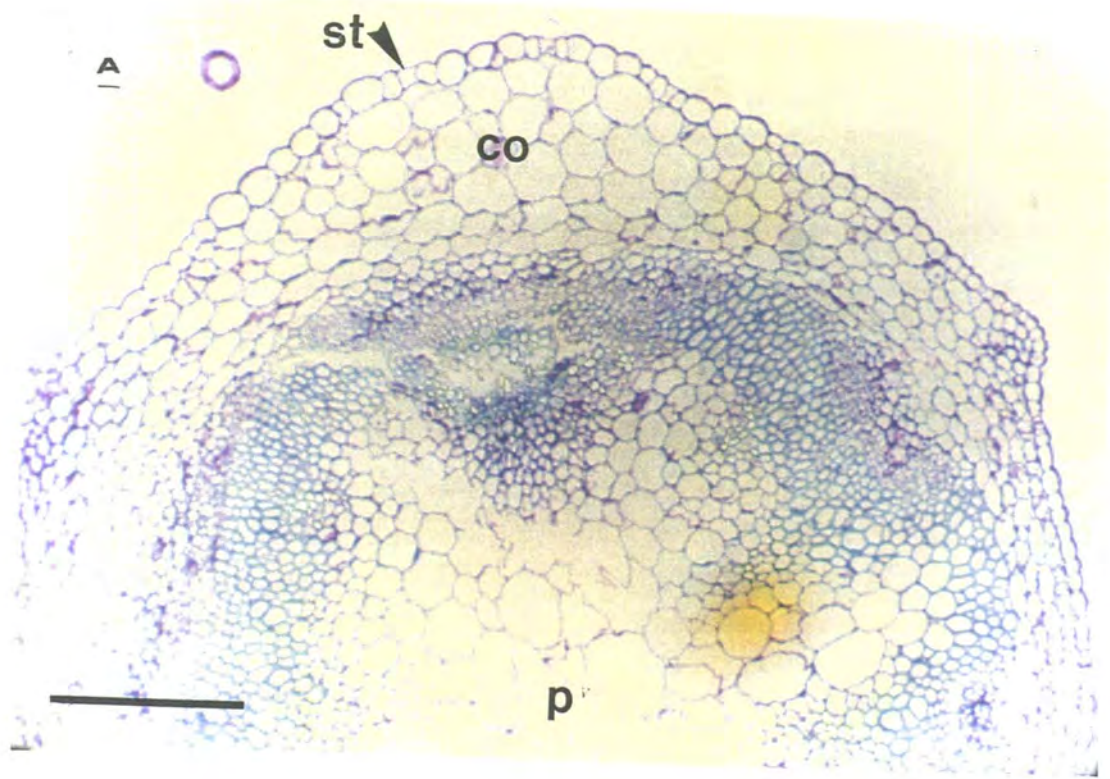


Plate 8.24 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under ZnSO_4 -stress conditions (100 ppm). ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm).

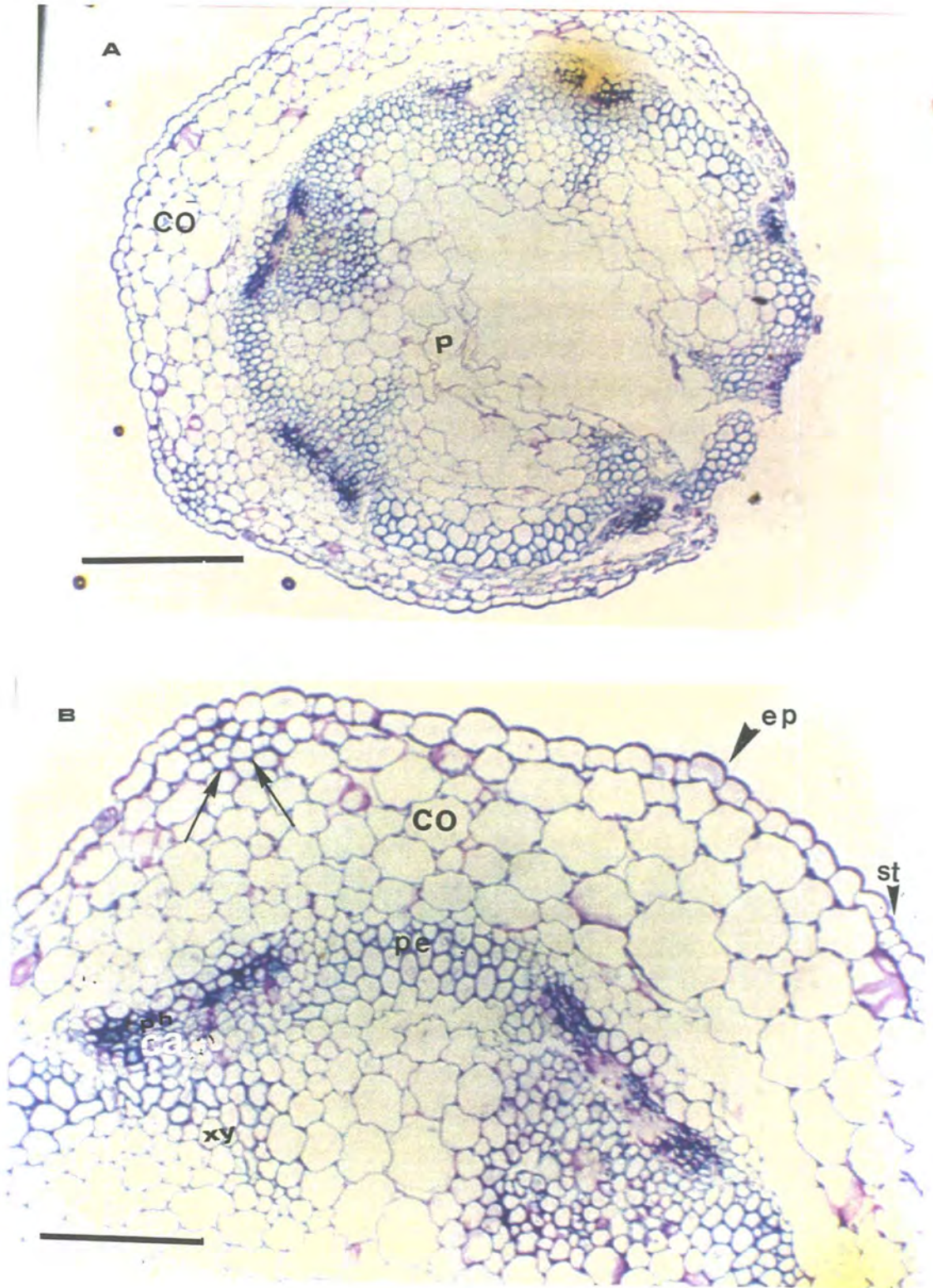


Plate 8.25 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under $ZnSO_4$ -stress conditions (200 ppm).

Plate 8.25 (A). Notice certain disrupted regions in stem.

Plate 8.25 (B). Notice a group of unusual cells in cortex (arrowed), larger cortex cells devoid of chloroplasts & lignified pericycle.

ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm).

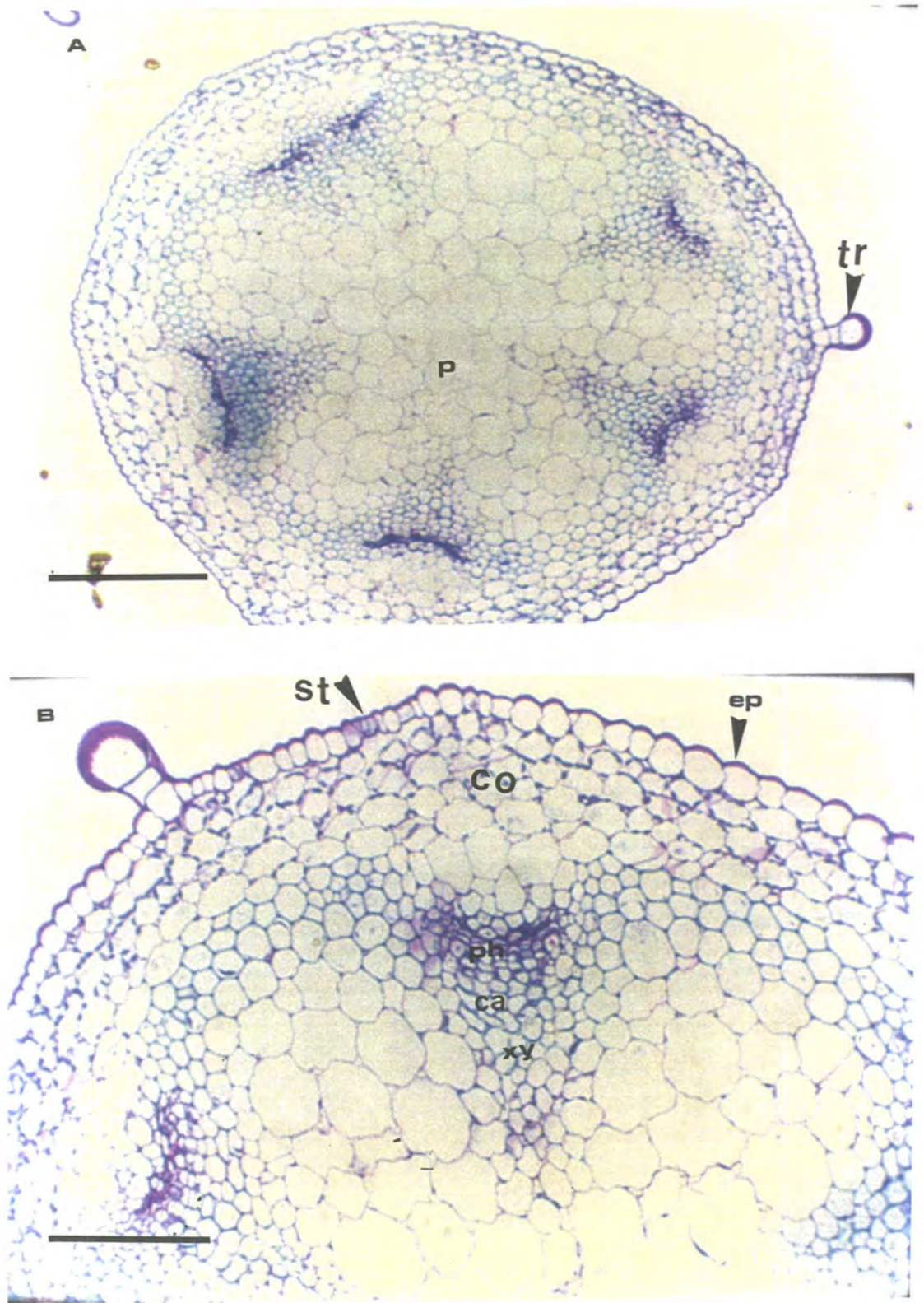


Plate 8.26 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under $ZnSO_4$ -stress conditions (300 ppm). Plate 8.26 (A). Notice slight change in the stem shape. tr, trichome; ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μ m, B = 50 μ m).

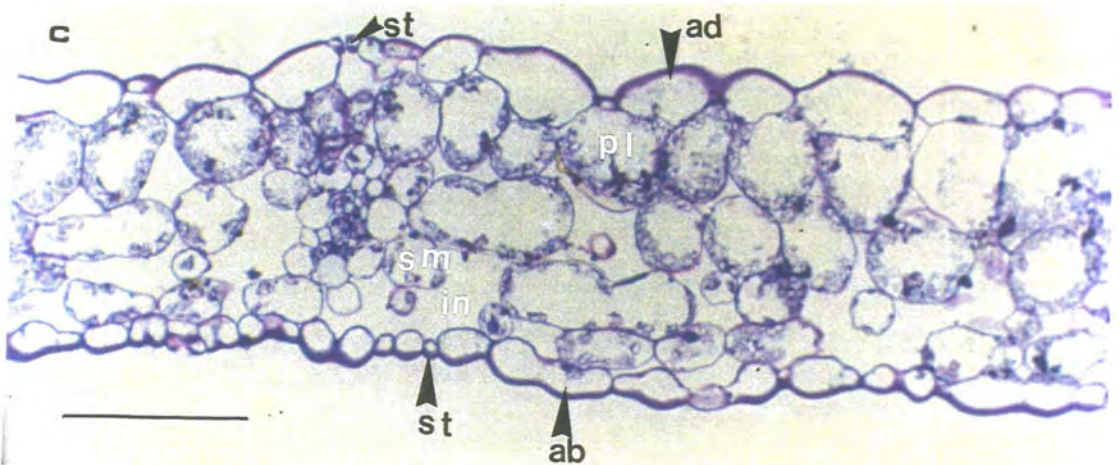
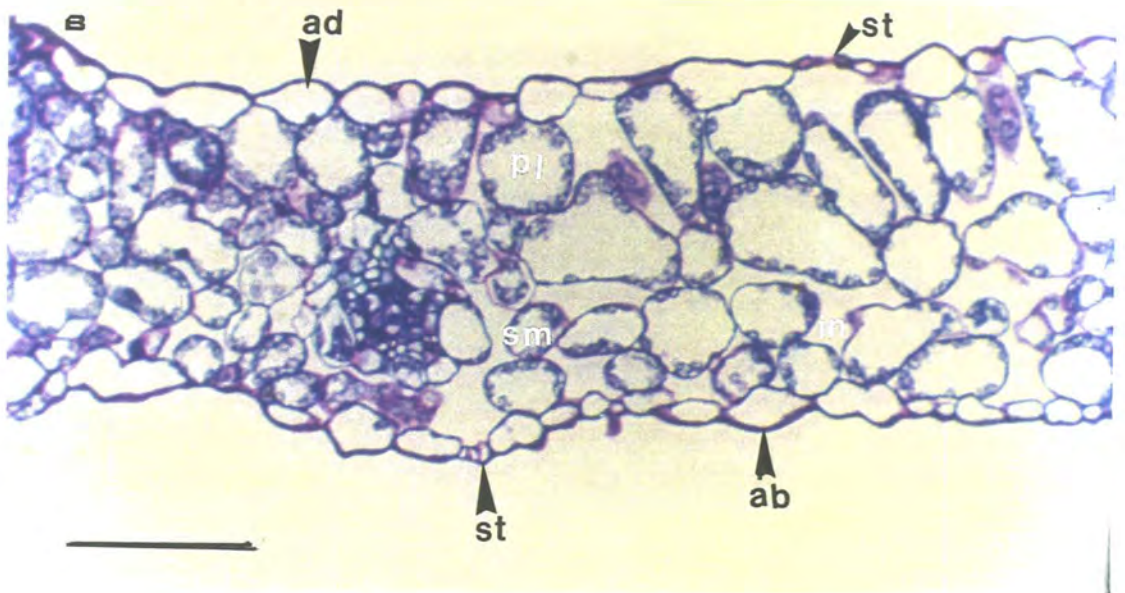
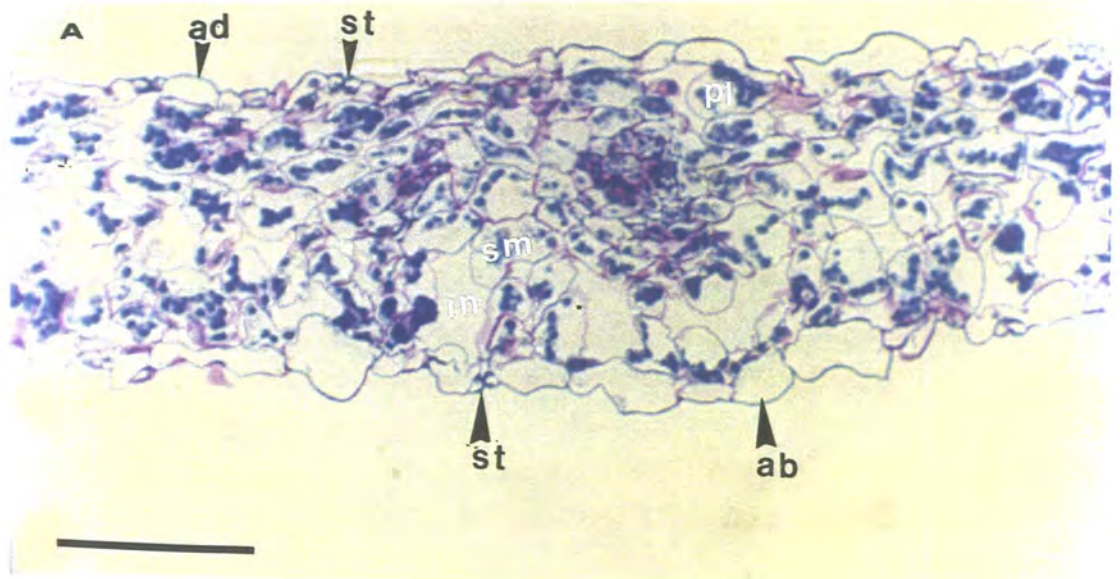


Plate 8.27 A-C Light micrographs of transverse sections through leaf of *Arabidopsis thaliana* (WT) seedlings, raised under ZnSO_4 -stress conditions A: 100; B: 200 ppm; C: 300 ppm.

Plate 8.27 (B). Notice bigger palisade parenchyma, spongy mesophyll cells with larger intercellular spaces and more chloroplasts.

Plate 8.27 (C). Notice larger palisade parenchyma and spongy mesophyll cells with large intercellular spaces.

ad, adaxial epidermis; ab, abaxial epidermis; pl, palisade parenchyma; sm, spongy mesophyll; in, intercellular spaces; st, stomata. (Scale bars = 50)

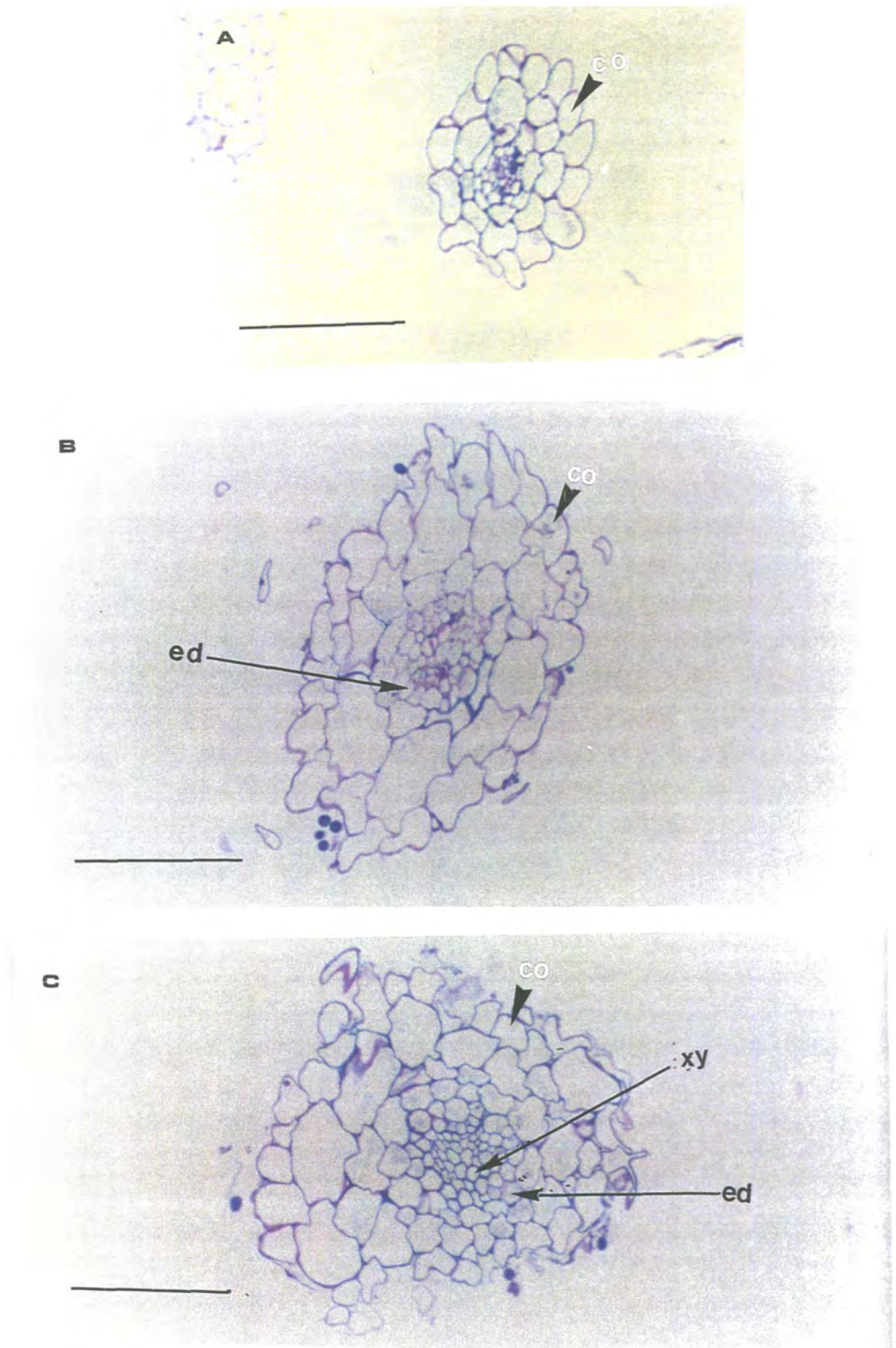


Plate 8.28 A- C. Light micrographs of transverse sections through root of *Arabidopsis thaliana* (WT) seedlings, raised under ZnSO₄-stress conditions A: 100 ppm; B:200 ppm; C: 300 ppm. co, cortex; ed, endodermis; xy, xylem. (Scale bars = 50 μm.).

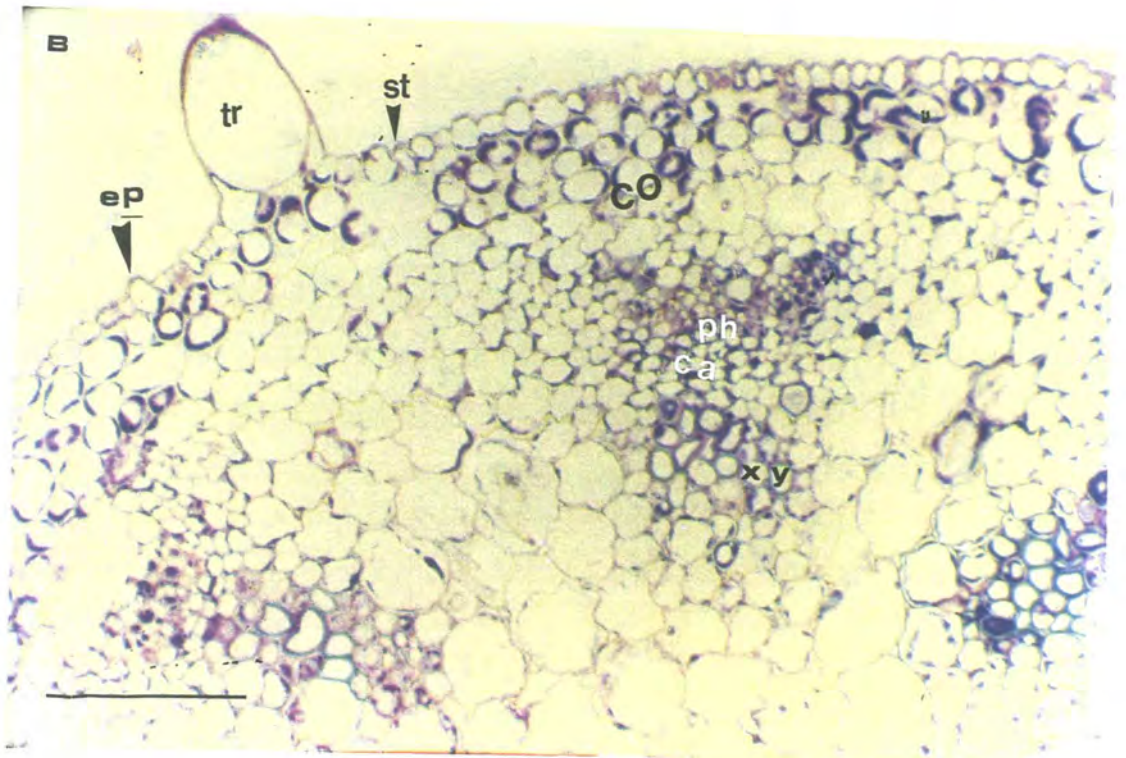
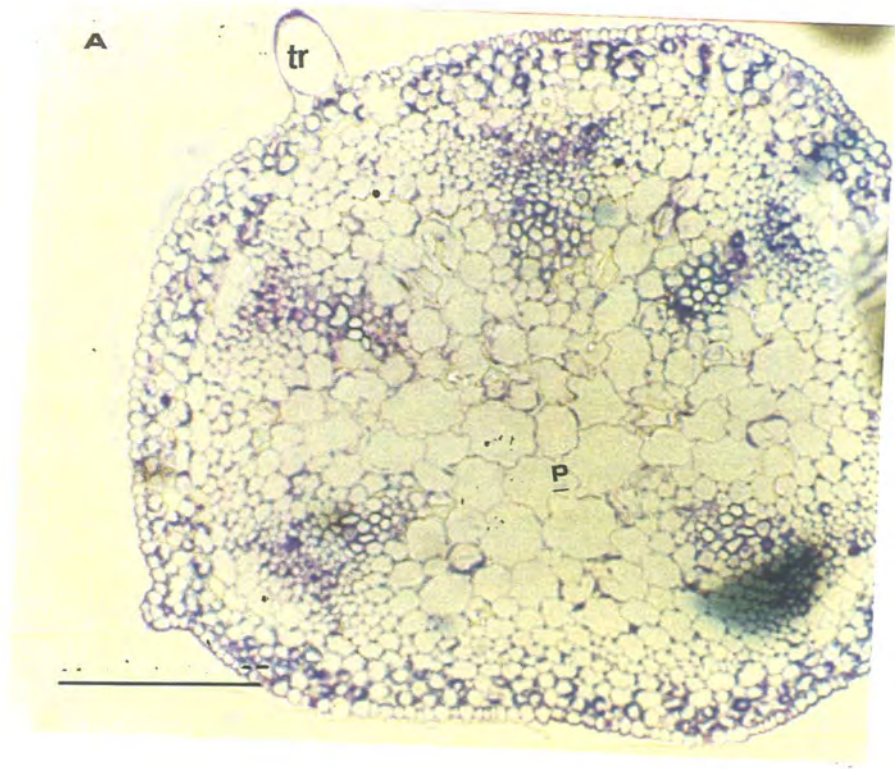


Plate.8.29 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under CuSO_4 -stress conditions (10 ppm). tr, trichome; ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm).

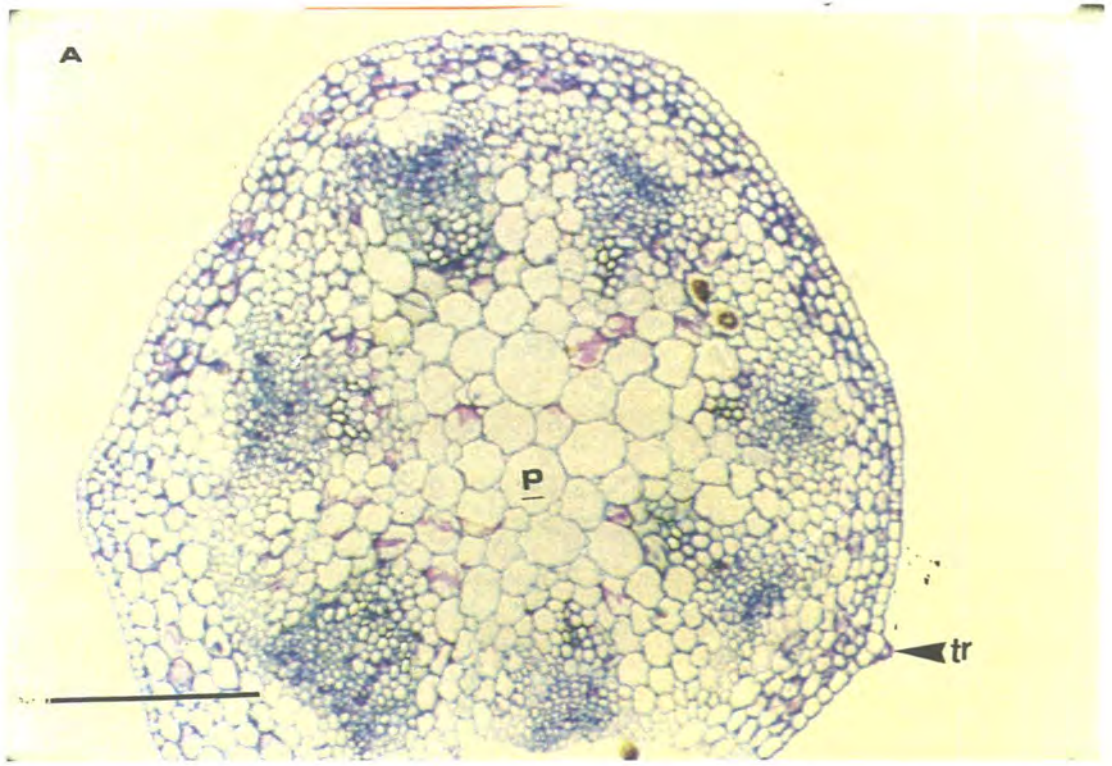


Plate.8.30 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under CuSO_4 -stress conditions (15 ppm).

Plate 8.30 (A). Notice slight change in stem shape.

Plate 8.30 (B). Notice relatively larger cortex cells and more lignified xylem cells than control. tr, trichome; ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm).

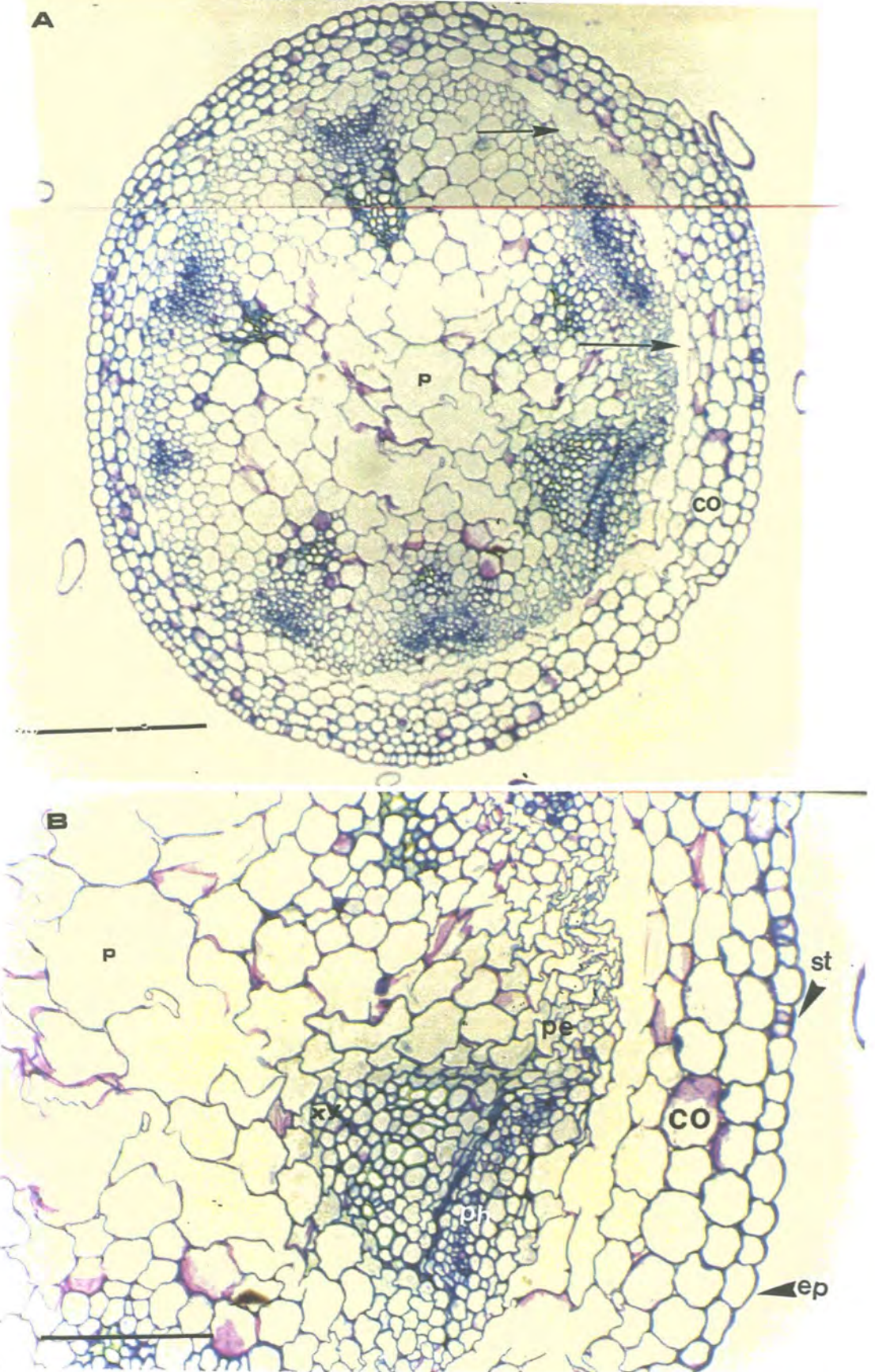


Plate.8.31 A- B. Light micrographs of transverse sections through stem of *Arabidopsis thaliana* (WT) seedlings, raised under CuSO_4 -stress conditions (20 ppm).

Plate 8.31 (A). Notice certain disrupted stem region (arrowed).

Plate 8.31 (B). Notice larger epidermal and cortex cells, disorganized pericycle and pith cells. ep, epidermis; st, stomata; co, cortex; pe, pericycle; ph, phloem; ca, cambium; xy, xylem; p, pith. (Scale bar, A = 100 μm , B = 50 μm).

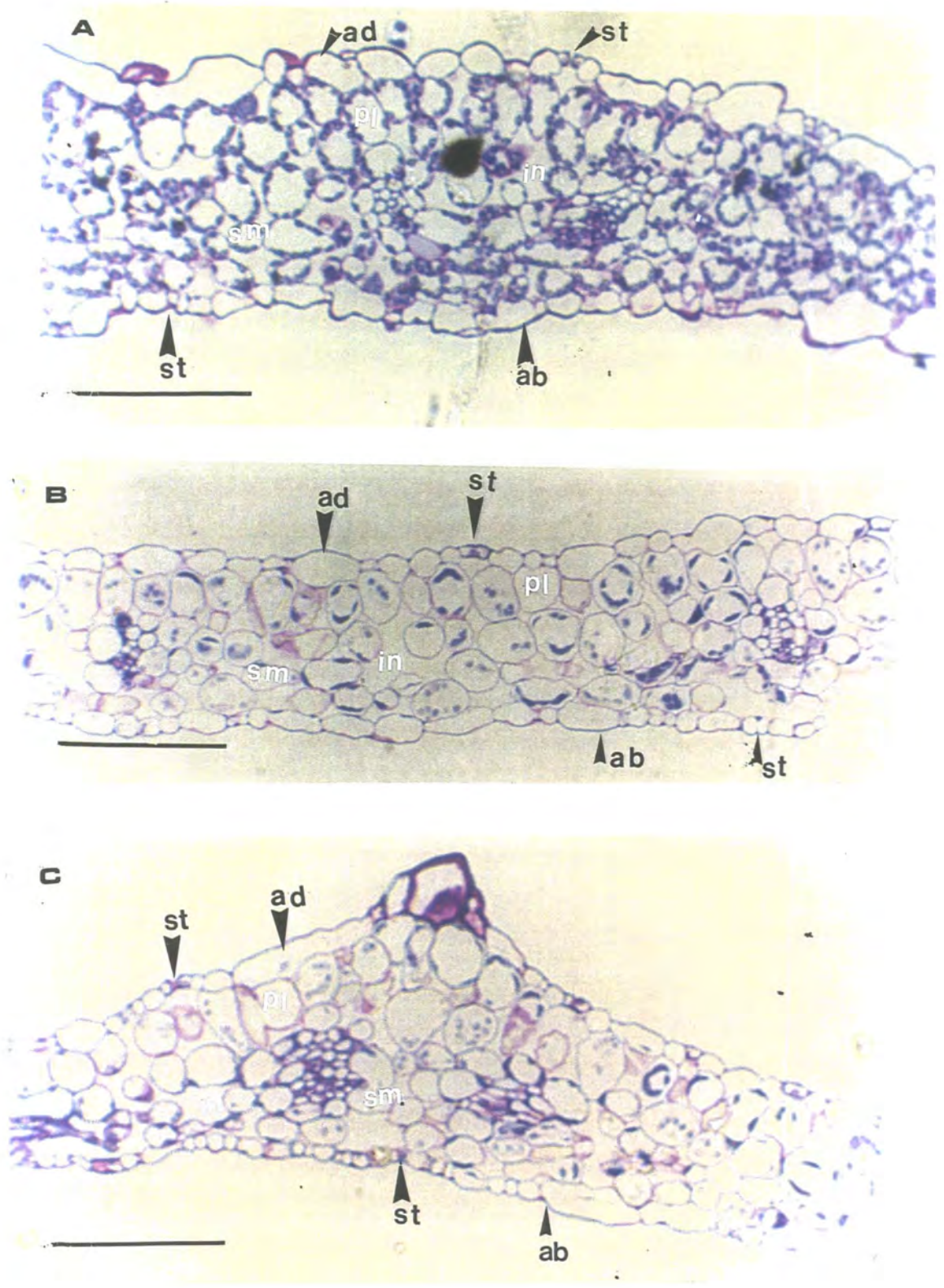


Plate 8.32 A- C. Light micrographs of transverse sections through leaf of *Arabidopsis thaliana* (WT) seedlings, raised under CuSO_4 stress conditions A: 10 ppm; B: 15 ppm; C: 20 ppm. ad, adaxial epidermis; ab, abaxial epidermis; pl, palisade parenchyma; sm, spongy mesophyll; in, intercellular spaces; st, stomata. (Scale bars = 50 μm).

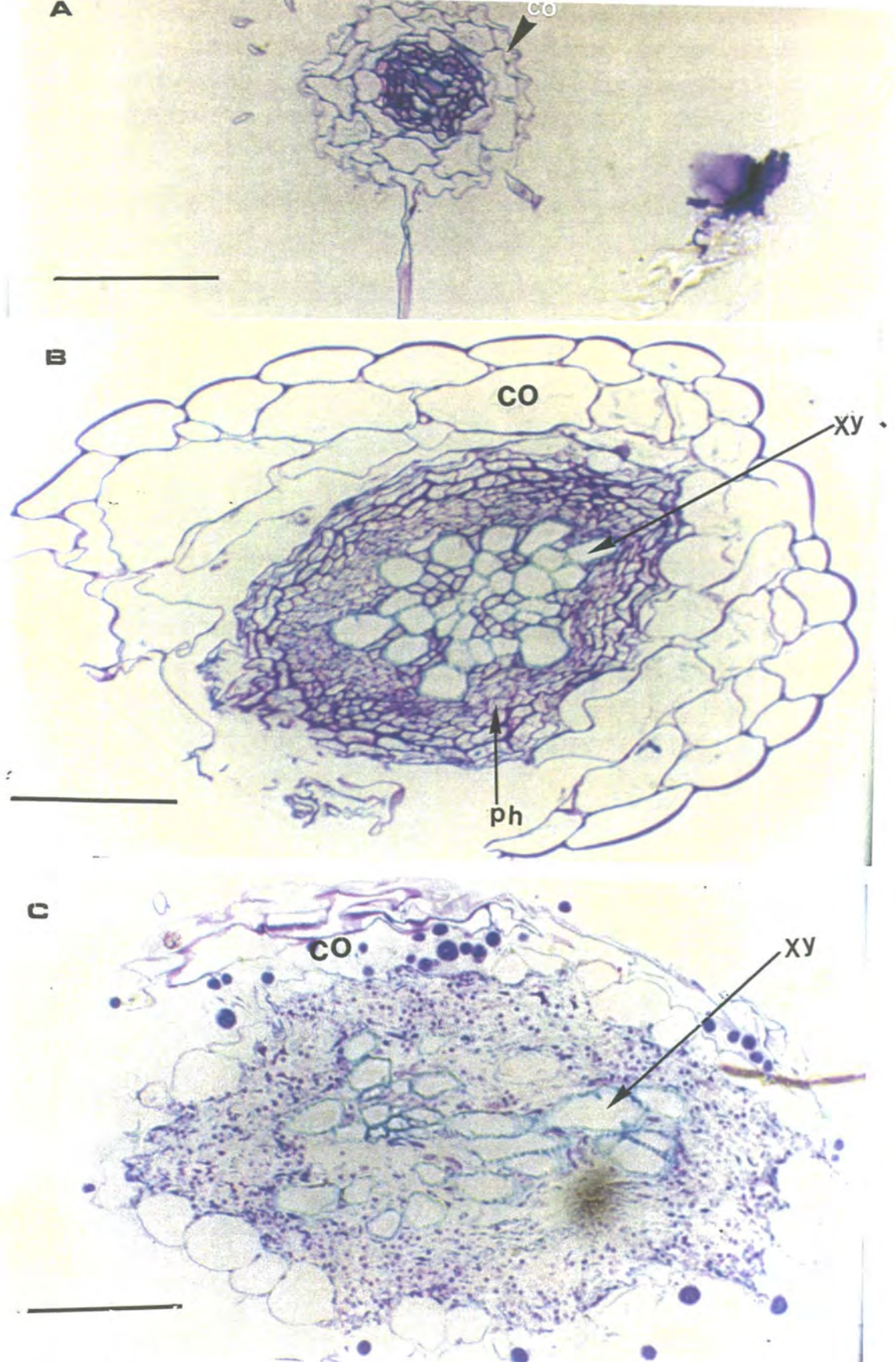


Plate 8.33 A- B. Light micrographs of transverse sections through root of *Arabidopsis thaliana* (WT) seedlings, raised under CuSO_4 -stress conditions A:10; B: 15 ppm; C: 20 ppm

Plate 8.33 (B). Notice disrupted cortical layers, endodermis & larger xylem cells than control.

Plate 8.33 (C). Notice disrupted cortex and endodermis & larger xylem cells.

co, cortex; ph, phloem; xy, xylem, (Scale bars = 50).

Chapter IX

DISCUSSION

9.1 Formulating pollen germinating medium

In order to formulate an effective pollen germinating medium for *Arabidopsis thaliana*, four mineral elements and in six concentrations with selected level of sucrose total of 21 media were prepared (Table-3.1). The results obtained are discussed below.

9.2 Effect of different media on pollen germination and growth

Examination of Table-3.1, showing different levels of mineral elements, and results in Table-3.3 indicate that there was little difference in germination when calcium nitrate level was raised from 0 ppm (44.38%) to 100 ppm (48.87%), but when it was raised to 200 ppm, the germination increased to 80.01% and increase in germination varied from 38.92% to 44.53% above M-2 and M-1 respectively. When this concentration was increased from 200 ppm to 500 ppm it exhibited an inhibitory effect on pollen germination from 40.41% (M-4) to 89.75% (M-6) in comparison with M-3.

From the results (Table-3.3) it was also concluded that as *Arabidopsis* pollen gives germination and tube growth in the absence of calcium this element therefore does not seem to be essential for these functions but is highly beneficial when added to the medium at the optimum level.

Turning to the effect of boric acid on PG and PTL (M-7 to M-11 including M-3), it was observed that from 0 ppm (47.35%) to 500 ppm (36.44%) excluding the 100 ppm, there was little difference in pollen germination percent between boric acid levels. The pollen germination was dramatically increased at 100 ppm in M-3 (80.01%).

For pollen tube length, there seemed to be a big variation in tube growth among the levels of boric acid, ranging from 442.24 μm (M-7, 0 ppm) to 185.0 μm (M-11, 500 ppm).

While studying the effect of boron it was observed that at 0 ppm many pollen grains and pollen tubes burst.

The data in Table-3.3 indicate that with no added boron in medium M-7, 47.35% pollen germination and pollen tube length of 442.24 μm suggests that, like calcium (as already discussed), boron also seems to be non essential for the germination of *Arabidopsis* pollen, but is highly beneficial when it is added to the medium at optimum level. These results are also in the agreement with the findings of Chiang (1974), who obtained similar results while working with cabbage (*Brassica oleracea* var. *capitata*) pollen.

As earlier workers including Visser (1955) and Vasil (1964b) reported, pollen grains can tolerate concentrations of boron up to 1200 ppm but optimum stimulation of germination was obtained at 10-150 ppm, depending upon species, growing conditions and endogenous levels of boron and borax available to the plant. Comparing the results obtained in the experiment under discussion, the best level of boron (100 ppm) was in the optimum range described by the above workers. It would be interesting to know how much endogenous boron is available in *Ara-*

bidopsis pollen, how it effects the pollen germination and growth and what is its correlation with exogenous supply of boron through germinating media. It was not within the scope of these studies to answer these questions, however.

While studying the effect of boron it was also found that at 0 ppm many pollen grains and pollen tubes burst. The excessive bursting of pollen grains and pollen tubes so often encountered in the absence of boron from the nutrient media may be due to a close negative correlation between tissue hydration and supply of boron, retarding deposition of new wall materials in the growing tip of pollen tube. The rapid cell enlargement resulting in the swelling of the pollen tube might either be due to excessive water uptake or to the mechanical weakness of the pollen tube wall. Rapid water intake has been observed in boron-deficient pollen (Gauch and Duggar, 1954).

O'Kelly (1957) reported that boron exercises a stimulatory effect on oxygen uptake and sugar absorption, besides having a specific role in pollen tube growth. Pollen tube growth stimulation under boron may also involve synthesis of the pectic materials of the growing pollen tubes or the synthesis of proteins.

Table-3.3 shows the effect of magnesium sulphate and potassium nitrate on the pollen germination behaviour and tube growth. Both of these mineral elements showed variable effects. Their varying concentrations in germinating media disturbed the mechanism of germination and tube growth at every level (except at the optimum level i.e. 100 ppm). Magnesium sulphate was most harmful at 500 ppm and the PG and PTL were also drastically affected in its absence. Potassium sulphate had a drastic inhibitory effects at 400 and 500 ppm. Like magnesium sulphate its absence from the medium affected the PG and PTL adversely.

Brewbaker and Kwack (1964) working with pollen of various plant species remarked that the *Ca* ion was essential for optimum pollen tube elongation. Its activity is directly a function of cationic balance in which *K* and *Mg* ions play most prominent roles.

The results also suggested that the *K* had more inhibitory effects on pollen of *Arabidopsis* than *Mg* in respect of pollen germination and pollen tube growth.

9.3 Effect of sucrose

Different levels of sucrose caused variations in the results (Table-3.4) recorded for pollen germination and pollen tube length. The PG and PTL were most affected by absence of sucrose and by high concentrations at 35% (w/v). During observations it was noticed that pollen grains and pollen tubes generally burst at 35% (w/v) sucrose concentration and in the absence of sucrose. The best response for pollen germination and pollen tube length was at 12% (w/v) of sucrose.

The results indicate that the PG & PTL were inhibited by both extreme levels of sucrose i.e. in absence of it and its highest concentration (35%, w/v). Both of these concentrations caused the large scale bursting of pollen grains and pollen tubes. The bursting of pollen grains and pollen tubes could be the result of either osmotic unequilibrium or nutritional imbalances. The issue has been the cause of controversy among earlier workers. One school of thought suggests that exogenous sugars are required only for osmotic control and not for nutritional purposes (Jost, 1905; 1907; Martin, 1913; Anthony, and Harlan, 1920; Visser, 1955). Another group of workers support the idea that apart from having an osmotic role, the externally supplied sugars, whether *in vivo* or *in vitro*, serve as an important source of nutrition (Tischler, 1947; Brink, 1924a; O Kelley, 1955, 1957; Hellmers

and Machlis, 1956; Vasil, 1960a,b, 1962b, 1964b; Johri and vasil, 1960, 1961; Kessler et al., 1960; Hrabetova and Tupy, 1963; Malik et al., 1982).

Considering the results in the light of above literature, it seems likely that if sucrose only had a nutritional function there would be no reason for poor germination and growth at 35%, the highest concentration provided in medium. This suggests that the role of sucrose for pollen germination and pollen tube growth is a two-fold i.e. osmotic regulation and nutritional.

9.4 Effect of Tris-HCl

As shown in Tables-3.10 and 3.12, the highest pollen germination and tube length was obtained at 25 mM, but when the level was increased to 50 mM and 75 mM pollen germination and growth drastically declined. This shows that when buffering the pollen germinating media, care should be taken to use the optimal concentration of buffer in such a way that it should not disturb the pollen germination and growth but should only balance the pH level while the process of germination and cell elongation is taking place.

9.5 Effect of pH

Non-significant differences among pH ($P \leq 0.05$) levels in respect of pollen germination and growth were obtained. This indicates that the PG and PTL of *Arabidopsis* are unaffected by the fluctuations in pH in the range of 7.0 to 9.0. The results are in agreement with the findings of Berg (1930), Branscheidt (1930), King and Johnston (1958) and Goss (1962), who showed that in many plants satisfactory germination of pollen takes place within a pH range of 4-9. The results are in disagreement with the findings of Stanley and Lichtenberg (1963) in which

they stated that the optimum pH for germination and growth in media containing borate compounds is generally between 6.5 to pH 7.1. Double-optima curves (two-peaked), expressing the percentage Double-optima curves (two-peaked) expressing the percentage of germination in a wide range of pH, were reported for several plants by Berg (1930) and Branscheidt (1930).

Vasil and Bose (1959) did not find any change in the pH of the medium even after pollen tubes had grown in it for about 2 hours. Vasil (1987) reviewing the findings of previous pollen researchers on the possible effects of pH on the pollen of different species could not draw any concrete conclusion, as to how pH affects pollen germination and growth. Opinions are so conflicting that it is difficult to decide whether pH influences the germination of pollen at all (Vasil and Bose, 1959). However Vasil (1987) in his concluding summary of pH effects on pollen, speculated that it is difficult to believe that an active metabolic phenomenon involving several enzymes in the uptake and utilization of carbohydrates and other nutrients, and the active growth of pollen tube wall, could remain virtually unaffected by the pH of the medium in so many plants, in spite of its expected effects on the availability of cations and anions present in the medium.

9.6 *In vivo* and *in vitro* comparison of pollen growth

The *in vivo* increase over *in vitro* in terms of percentage (Table-3.13) varied from 78.78 to 83.49%.

The results add support to findings of some early workers. They showed that the rate of pollen tube growth *in vivo* is in the range of 1500-3000 $\mu\text{m}/\text{h}$ in species, with a maximum of 7500 $\mu\text{m}/\text{h}$ (Buchholz and Blakeslee, 1927; Brewbaker and Kwack, 1964). Poddubnaja-Arnodi and Dianowa, (1964) reported an incredible

rate of 35000 $\mu\text{m}/\text{h}$ for *Taraxacum kok-saghyz*. In comparison pollen tubes growing *in vitro* in *A. thaliana* elongate at the rate of only 36-50 $\mu\text{m}/\text{h}$.

9.7 Genotype response towards standard medium (SM)

The results depicted in Table 3.14 indicate that the highest pollen germination given was by the genotype NW-45 relating to the Landsberg group, and the highest pollen tube length by 25/60, M2 with the subpopulation of the Columbia group. From this result it can be assumed that pollen germination and pollen growth could be genetically independent controlled characteristics regulated by independent sets of genes. With the exception of the wild type, all other genotypes tested were mutants (assumed to be single gene mutants), so differences observed in respect of PG and PTL could be the result of some mutant genes, which have phenotypic effects that are expressed in the general morphology of the plant and in the physiological responses of its gametophytes.

9.7.1 Comparison between the pollen germination and growth of *A. thaliana* and *C. arietinum*

Some plant species contain binucleate pollen, some trinucleate. In the present studies two plant species, *A. thaliana* with trinucleate and *C. arietinum* with binucleate pollen, were selected to compare their PG & PTL under the mineral nutrient conditions described in the materials and methods (above).

Trinucleate pollen are considered to be difficult to germinate *in vitro*, requiring complicated pollen germinating media, but binucleate pollen are comparatively easy to germinate. *A. thaliana* pollen required a pollen germinating medium which included H_3BO_3 , $Ca(NO_3)_2$, $MgSO_4$, KNO_3 and sucrose, while *C. arietinum*

pollen germinated in boric acid, calcium nitrate and sucrose.

The best pollen germination and pollen tube length in *A. thaliana* pollen was recorded with 100 ppm of H_3BO_3 and in *C. arietinum* the best PG & PTL was recorded with 1000 ppm of H_3BO_3 . This result indicates that optimum H_3BO_3 requirement of *C. arietinum* pollen for its germination and pollen tube growth are relatively 10 times more than *A. thaliana* pollen.

Comparing the calcium nitrate requirements, the data depicted in Tables 3.3 show that *A. thaliana* pollen gave maximum pollen germination (80.01%) and pollen tube length (750.65 μm) in 200 ppm of calcium nitrate. *C. arietinum* produced highest growth with 300 ppm of calcium nitrate. This result also shows that the calcium requirements of *C. arietinum* in respect of its pollen germination and growth are higher than *A. thaliana* pollen.

Evaluating sucrose requirements of both species for PG & PTL, it was recorded that *A. thaliana* exhibited maximum PG & PTL with the addition of 12% (w/v) sucrose. The *C. arietinum* pollen germinated best with 8% (w/v) sucrose. Its highest pollen tube length was obtained at 6% (w/v) sucrose. This shows that the sucrose (osmotic/nutritional) requirements of *A. thaliana* for PG & PTL are different from those of *C. arietinum*.

Both species under discussion have different nutritional requirements for their pollen germination and growth. The results from the experiment conclude that *in vitro* pollen germination and pollen tube growth requirements are likely to be species specific, so it is unlikely to be possible to make broad generalizations about pollen mineral nutrient and osmotic requirements for all species.

9.8 Effect of temperature on pollen germination and growth in *Arabidopsis*

.....a close study of plant & animal life offers us the best means of monitoring the effects of climate change. Even minor fluctuations in temperature have an impact on their patterns of growth & behaviour. A small change in temperature has the same effect on plants as an increase in interest rates on the economy of a nation. The effect is cumulative over a period of time & although the change may be small, the net effect at the end of accounting period can bring ruin or salvation.

(PHILLIP GATES)

The results (Table-4.1) indicate that the pollen germination was affected variably by the different temperature regimes in comparison to the control (20°C) in the case of all the three genotypes tested. The germination was more adversely affected under both extreme temperatures i.e. 0°C and 35°C.

The results obtained for pollen tube length (Table-4.2) also indicate that like pollen germination the pollen tube length was also adversely affected under lowest (0°C) and highest (35°C) incubated temperature regimes in all genotypes in comparison to the control (20°C)

The results found in this study for pollen germination and pollen tube length add the support to the findings of Roberts and Struckmeyer (1948), Visser (1955) and Vasil (1962b) who recorded the harmful effects of temperature regimes in different plant species.

During observations some clear effects of temperature were noted on pollen tubes. At 0°C and 5°C the rate of growth was very slow. Such reduced rates of

pollen germination and pollen tube growth were observed by Smith and Cochon (1935) at low temperatures. At 10°C and 15°C branching and abnormal growth of pollen tubes was observed and at 30°C bursting of tubes commonly occurred. Such results confirm those of Vasil and Bose (1959), and Vasil (1962b), who recorded similar effects of different temperature regimes in various plant species.

9.9 *In vitro* rate of pollen germination and growth in *Arabidopsis*

The results (Table-4.3, 4.5) indicate that the germination and pollen tube growth rate were very slow up to the 3rd hour, and enhanced from the 3rd to 15th hour and then gradually decreased. Such results are in accordance with the findings of Vasil (1960a, 1962b). He described growth curves of pollen tubes growing *in vitro* as being typically sigmoid, for a number of species. Such growth curves are characterised by a brief period (lag phase) of slow growth at the beginning, followed by a long period of rapid elongation, and finally a period of gradual decrease in the rate of growth which ends in the cessation of tube elongation.

As indicated in Tables-4.4 & 4.6 that the highest calculated *in vitro* pollen germination and pollen tube growth rates of were obtained at 20°C. The rate of pollen growth was strongly affected by temperature regimes.

9.9.1 *In vivo* and *in vitro* comparison under different temperature levels

The results shown in Table-4.7, indicate that the range of temperatures tested (10°C-30°C) had no significant difference on pollen growth *in vivo* and *in vitro*. But the superiority of *in vivo* pollen tube growth over *in vitro* found in these studies is in accordance with the work of many earlier pollen workers, like Buchhoiz and Blakeslee (1927), Brewbaker and Kwack (1964), Poddubnaja Arnoldi and Dianowa

(1934) who working with different plant species recorded longer pollen tube lengths *in vivo* than *in vitro*.

9.9.2 Effect of temperature on pollen germination and growth in *Cicer arietinum*

Comparing the pollen germination and pollen tube length of *C. arietinum* with PG & PTL in *A. thaliana* (wild type), it was noticed that (Table- 4.1, 4.2, 4.8) *A. thaliana* produced greater PG & PTL than *C. arietinum* at higher (25-30°C) and lower (10-15°C) temperatures. This suggests that *A. thaliana* pollen may have more temperature adaptability than the *C. arietinum* pollen, an observation which correlates with the climatic conditions in the natural geographical ranges of these species.

9.10 Impact of differential pollen growth rates on the sporophytic traits of *Arabidopsis thaliana*

To investigate whether the differential pollen tube growth rates have selective effects on ovule fertilization, seed development and sporophytic traits, an experiment with four genotypes, having differential pollen growth rates, was devised. The results obtained are discussed below.

9.10.1 Crosses

The results obtained (Table-5.1) showed consistency in the relationship between pollen donor growth rates and successful fertilization of ovules resulting in development of seed. The consistent ranking of relative donor growth rates is in accordance with the hypotheses that they result from genetic, rather than environmental factors. Whether these growth rates are heritable, however remains to be demonstrated (Chasan, 1992). While conducting the experiment for salinity

stresses (chapter-6), the effect of environment (salt) stresses on pollen growth rates on one of the *Arabidopsis* genotypes (wild type) was recorded. Highly significant results ($p \leq 0.001$) were obtained (Table-6.1-6.2) for pollen growth rates, when either the pollen was collected from salinity stress-free plants and germinated in the salt supplemented pollen germinating media, or pollen was collected from salinity stressed plants and germinated in non supplemented salt pollen germinating medium. These results demonstrate that the environment also seems to play a vital role in varying the pollen growth capabilities of the plant species. For a further discussion of these results, see page 274-275.

The results recorded are in agreement with findings of Jones (1928), who mentioned that differences in the fertilization ability were due to differences in pollen tube growth rates. Ottaviano et al. (1975) also found differing fertilization abilities attributable to variation in pollen tube growth rates among strains of *Zea mays*.

The results are in disagreement with the findings of Mazer (1987) who, using single pollen donors, found no significant additive genetic variance in male performance for ovule fertilization, ovule growth, number of seed/fruit, or seed weight/fruit in *Raphanus raphanistrum*.

It is likely that the variations in PTGRs may be the result of a combination of environmental and genetic interactions. The relative importance of each source of variation remains to be determined.

9.10.2 F1 hybrids

In order to study the effect of pollen growth rates on sporophytic traits, the seeds of each cross were collected separately and the F1s were raised and studied.

Comparing the data with controls (Table-5.2), in every trait there was either an increase or decrease in vigor against controls for different pollen donors. But the remarkable peculiarity of the data is that almost every characteristic exhibited a consistent increase over controls in the case of the genotype CAD1, having very high pollen tube growth rates (615.78 μm). Its vigor for various traits varied from 0.67% (B/P) to 114.82 % (PDW) and from 0.75% (FF) to 134.19 % (PDW) in the cases of pollen donors and F1s respectively. Only in one of the parameters of earliness (days taken from raceme to flowering) did it show a decrease, of 10.34 %.

The results obtained showed straightforward increases for most of the sporophytic traits in the case of genotype CAD1, having highest pollen growth rates (Table-5.2, 5.3). The results therefore indicate that the sporophytic plant characteristics are highly influenced by the parental PTGRs.

The consistency and the stability of the wide range of sporophytic characteristics in F1s sired by the fastest pollen donor (CAD1), supports but does not prove the concept that speed of the pollen growth is positively correlated with the quality of resultant sporophytic generation and pollen tube growth rates may be used to predict the quality of F1 crosses in crop species (Ottaviano et al. 1980), i.e. to predict the combining ability.

The view that selection among pollen is important in plant evolution assumes that pollen genotypes with fast pollen tube growth rates tend to produce progeny with high fitness (Mulcahy and Mulcahy, 1975, 1987; Mulcahy, 1979). Differences in pollen tube growth rates have been found among various strains of inbred cultivated plants, or produced by selecting for fast growing pollen tubes (Pfahler, 1967; Mulcahy, 1971; Sari Gorla et al., 1975; Johnson and Mulcahy, 1978; Otta-

viano et al., 1980, Gawel and Robacker, 1986). Similar differences in pollen growth rates between plants from different populations could be due to genetic variation (Stebbins, 1950; Charlesworth et al. 1987).

In quantifying the vigor of hybrids against pollen donors (Table-5.4), no generalized conclusion could be drawn for all of the sporophytic traits under discussion and PTGRs. In the case of the cross MS x NW-46, declines of 41.73% and 2.30% were obtained for PTGRs and pods/plant respectively. Other traits increased from 23.94% (plant height) to 66.67% (branches/plant).

In the case of the cross-combination MS x NW-28, increases of 5.75% (plant height) and 78.65% (pods/plant) were recorded, while the remaining sporophyte traits declined by 1.23% (rosette diameter) and 34.82% (branches/plant). The PTGRs declined by 31.37%.

In the case of the hybrid MS x NW-91, this cross produced a positive performance increase over its pollen donor parent in respect of PTGRs and sporophytic traits.

In the cross MS x wild type, all hybrid traits performed negatively in comparison to paternal parent.

In the combination MS x CAD1 only branches/plant increased (6.04%) relatively with pollen donors and there was a decline in PTGRs and the rest of the sporophytic traits.

The performance of a particular cross may deviate from the average of the two parental lines; this deviation is known as the specific combining ability (SCA) of that cross (Falconer, 1960). The result indicates that possibly no generalization

could be predicted for the hybrid vigor in F₁s. Hence the result concludes that specific combining ability obtained in F₁s may be specific for particular traits and particular genotypes and not simply related to PTGRs.

9.10.3 Correlations

The correlations between gametophyte and sporophyte traits were evaluated. The parameters used for earliness either gave negative or very loose correlations. This shows that perhaps earliness of plants is either genetically more stable or the gametophytic and sporophytic genes for these traits do not overlap in both stages of plant life cycle. Most of the agronomic characteristics taken into account indicated positive correlations.

EFFECTS OF SALINITY (*NaCl*) ON THE SPOROPHYTE AND GAMETOPHYTE OF *A. thaliana*.

Experiment 1

9.11 Male-gametophyte: an index for prediction of salinity limits and selection for salt tolerance in plant species

9.11.1 Impacts of salinity on gametophyte

The results depicted in Table-6.1 & 6.2 show the similarity of *NaCl* effects on the gametophyte of both plant groups, salinity stress-free and salinity-stressed plants. The pollen germination and pollen growth rates were affected similarly at all salinity levels, while pollen tube length to some extent responded differently. In pollen from stress-free plants, pollen tube length was stimulated at low salt levels (100, 500 ppm), but declined at high salt concentrations (1000, 2000 ppm). In the

case of salinity-stressed plants it was stimulated at all levels (500-5000 ppm) of added *NaCl*. The disparity in PTL of both groups may be due to the following reasons:

- (a). Nature of availability of saline conditions transmitted to them through the pollen germinating media to the stress-free plants and through plant transpiration system to the stressed plants.
- (b). Could be selective destruction of salt sensitive pollen in developing anthers of stressed plants. Selection occurring in the anther before pollen is shed.
- (c). Physiological effect, osmotic adjustment of pollen during its formation in anthers of saline-stressed plants.

The distinctive responses of pollen from both groups i.e. salinity stress-free and salinity stressed plants, could help researchers to predict the possible effects of salinity through gametophytic selection (see below) by quantifying the limits of environment effects during pollen formation or pollen salinity tolerance during germination and tube growth

The data (Table-6.2) indicate those changes that could be predicted in pollen of *Arabidopsis* when its sporophyte is subjected to the different percentages of salinity near the pollen lethal level (PLL) in pollen germinating medium. For instance, when plants of *Arabidopsis* were subjected to the 25 % (500 ppm) of PLL (2000 ppm), there was 1.96 % decline in PG with 17.35% and 16.65 % increases in PTL and PGRs respectively. In this way, the data indicate that at different added salinity stress levels against PLL, different changes occurred in PG, PTL & PTGRs. This type of information or data could be termed a plant pollen salinity index (PPSI).

The results confirm those of some earlier workers. The decline in pollen germination at all salinity levels is in accordance with the work of Ota et al. (1956), who demonstrated that a reduced number of fertilized florets in rice panicles under saline conditions is due to the possible effect of salts on pollen viability and germination. Abdullah (1978), while working on wheat showed that increasing salinity levels decreased pollen viability and germination.

9.11.2 Impacts of salinity on sporophytic traits

Striking differences for the various salt concentrations on the sporophytic characteristics (Table-6.4) were observed.

1. Effect of salinity on roots

The decline in the root length in these studies (Table-4.4) at all salinity levels seems to be contrary to the findings of Marcum and Mardoch (1992), who reported the stimulation of root of *Sporobolus virginicus* (Graminae) under saline solution cultures. These differences may be due to the use of different plant species; responses may be species-specific.

The primary effect of salinity is thought to take place in roots (Meizner, et al., 1991; Munns and Termaat, 1986). It has been argued that this primary effect is water deficit rather than specific toxic effect of salt *per se* (Munns and Termaat, 1986). In contrast to studies conducted with hydroponics, results from experiments using soil-grown plants have often shown at least as severe reductions in root as in shoot growth at elevated salinities (Bingham and Garber, 1970; Chavan and Karadge, 1980; Papadopoulos and Rundings, 1983; Zakri and Parsons, 1990; Snap, et al., 1991) This suggests that salinity effects on root systems can be significant

and may be affected by the growth media also.

2. Effect of salinity on the shoot

Highly significant results ($p \leq 0.001$) for plant height (PH), branches/plant (B/P) and nonsignificant results for shoot dry weight (SDW) were recorded. For all three characteristics (Table-6.3), stimulatory effects at lower levels of salinity were found, while increased salinity concentrations substantially reduced the PH, B/P and SDW. The stimulations at low salt levels are in accordance with the findings of Marcum and Murdoch (1992) and Flowers et al. (1977).

The plants during the present study were transferred to the hydroponic salt supplemented media at the flowering stage and were allowed to grow on to maturity. The inhibition in the shoot growth may be a long-term response, which results from an excessive accumulation of salt in leaves (Flowers et al., 1986; Yeo et al., 1991). This accumulation results in osmotic and ionic effects that both appear to be important in the long-term response to salinity.

3. Effect of salinity on fruiting parts

The fruiting parts of *Arabidopsis* were variably affected (Table-6.3, 6.4) by salinity. Pedicle length was nonsignificantly reduced at every level of salinity, while pod length was significantly ($p \leq 0.001$) stimulated at low levels and adversely affected by high salinity levels.

If the overall results are reviewed in the light of previous literature, the previous findings indicate that approximately 60% of genes expressed in the sporophytic generation of plant are also expressed in the gametophytic generation (Tanksley et al. 1981; Sari-Gorla et al. 1986, willing et al. 1988). Most of the genes expressed

in both of generations are supposed to participate in general metabolic functions (Brewbaker 1971; Ottaviano et al. 1980; Weeden 1986). Relating these findings to the present studies, it is clear that at mild or intermediate levels of salinity the pollen germination, pollen tube length and pollen tube rates were stimulated and at heightened salinity stress conditions these were inhibited. Generally similar conditions as recorded for gametophytes were observed for sporophytic traits at mild and high concentrations, with similar trends of stimulation and inhibition. Plant breeding programmes mostly include qualitative plant characters like disease resistance, cold or drought tolerance and mineral stress (Christiansen and Lewis 1982). These traits happen to be expressed in gametophytes also. For instance, gametophyte sensitivities paralleling those of the parent plant have been reported for salinity (Eisikowitch and Woodell, 1975), temperature (Herrero and Johnson 1980; Zamir et al. 1982; Zamir and Vallejos 1983; Weaver et al. 1985), ozone (Feder 1985), heavy metals (Searcy and Mulcahy 1985) and fungal toxins (Bino et al. 1988).

In the light of above literature we can conclude that the evaluation of gametophyte performance under stress would be of great help in planning the long-term research programmes for screening against stress conditions. Plant breeders can begin to formulate the Pollen Plant Salinity Index (PPSI) of particular species under specific growing conditions, and exploit it for plant improvement. This technique could not only be used to select against salinity but could also be a useful tool to use it for selection of tolerant ecotypes against heavy metals, high and low temperatures regimes, or any such other stress conditions that could be imposed on pollen germination *in vitro*.

Experiment 2

9.12 Fruit-setting, seed-setting and seed-filling in *Arabidopsis thaliana* in response to high saline conditions

The experiment was conducted to study the impact of single and double salinity stress conditions on the pollen to determine whether salinity stress during pollen gametogenesis preconditioned pollen to high salinity levels during germination *in vitro*, and to investigate the effect of salinity regime on fruit-setting and successful seed production. The germination of saline-grown pollen on saline medium (Table-6.6) was greater than the pollen of non-saline or stress-free grown plants. The greater germination percentage and tube length of saline versus control pollen on saline medium suggests that there may be physiological adaptation during pollen development (Sacher, et al. 1983).

In the case of fruit-setting, reductions of 8.13% to 11.67 % were observed in the fruit-setting capabilities of plants under salinity stress conditions (Table-6.7). When pollen came from the similar saline stress conditions to that which maternal parents were growing (MS-HS x wild-HS) the fruit setting was less affected, but if pollen and pistil had experienced differential saline conditions (MS-HS x wild-SF, MS-SF x wild-HS), the fruit-setting was more affected. Similarly seeds per pod and filled seed percentage was comparatively less affected if pollen came from plants grown under the same stress conditions as the female parent. The results suggest that there may be physiological interactions during pollen tube growth and fertilization when pollen and pistils are grown under similar stress regimes. Failure in fruit-setting and seed fertilization may be greater when there are dissimilarities in stress conditions of pollen and pistil.

Overall trend of salinity losses in the fruit-setting & seed-setting is in the

agreement of the previous literature that salinity showed adverse effects on corn grain production (Piruzyan, 1959), and on grain setting failure in rice in saline soils (Kapp, 1947). The results obtained in this studies add support to the findings of Sacher et al. (1983), who recorded more pollen germination of the saline grown pollen on the saline medium than pollen from non-saline or stress-free plants. From the results he concluded that greater pollen germination and tube growth of saline versus control (non-saline) pollen on saline medium suggested that there may be physiological adaptation during pollen development.

9.13 Anatomical changes brought about by salinity in the stem

leaf and root of *Arabidopsis thaliana*

Relatively little is known about the anatomical changes caused by the salinity stress conditions in the plant species. The results obtained during these studies regarding the changes brought about by the applications of salinity (*NaCl*) in the stem, leaf and root of *A. thaliana* are discussed as below.

9.13.1 Anatomical changes in stem

The photographs show the TS of the stem of control plants raised without saline conditions (Plate-6.1) and plants raised under saline conditions (Plate-6.2-6.4). The results show that the size of treated epidermal cells deviated from the control cells. Cells become elongated under 250 ppm *NaCl* (Plate-6.2) and even larger under elevated salinity levels. The cortical parenchyma cells became more elongated and rounded than the control, in the 250 ppm *NaCl* treatment. At 500 ppm the cortex contained more parenchyma cell layers with larger cells than the control. The innermost layer of cortical cells separated the stele and cortical region with irregular spherical and larger cells compared with the slightly cylindrical cells

of the control. Under 1000 ppm the cortex showed larger parenchyma cells than the control. The changes in the cortical region recorded in these studies are in the line with findings of Serrato et al. (1991), who reported that in *Prosopis tamarugo* the increase in stem diameter of saline grown plants (400 ppm) was correlated with a higher number of cortical cell layers.

The cortex at elevated levels of salinity contained little (Plate-6.4) or no chloroplast development. The decline in chloroplasts is not known. It has earlier been reported that certain heavy metals cause the disintegration of some chloroplasts in stems, Barcelo et al. (1988) working with the effects of *Cd* on the stem anatomy of *Phaseolus vulgaris* reported similar results. Similar results were also recorded by Wise et al. (1983) for water and chilling stresses.

The vascular system did not show any major change under the 250 ppm stress condition but under the 500 ppm *NaCl* (Plate-6.3) the cambium area was broader with slightly larger cells than the control. Changes in the vascular system were reported by Serrato et al. (1991), working with *P. tamarugo*, who reported that at 400 mM, the disorganization of vascular bundles was evident in stems with small xylem groups. Phloem elements and cambium were difficult to distinguish. The disorganization of the vascular system in studies described by Serrato et al. (1991) was not on such a scale. This may be due to either higher salinity doses or different plant species used by the above investigator.

Unexpectedly, except at 500 ppm *NaCl*- stress conditions, the pericycle was non-lignified. Otherwise under the remaining stress conditions the pericycle was lignified (Plates-6.2, 6.4). The xylem cells were also lignified, mostly with the continuous lignification with pericycle. The results recorded agree with the findings

of previous workers, who reported that the early lignification is frequently observed in halophytes and also nonhalophytes grown in saline conditions (Millner, 1934; Saadeddin and Doddema 1986; Solomon et al. 1986).

9.13.2 Anatomical changes in leaf

The results presented in Plates-6.5-6.6 indicate the changes created by the salinity in the leaf tissues of seedlings treated with different *NaCl*-stress conditions. The results show that salinity generally decreased the size of the epidermal cells in both adaxial and abaxial surfaces of the leaf, in comparison to the control. The effects of salinity on the epidermal cells is a controversial issue. Ivanitskaya (1962) working with *Gossypium hirsutum* reported the reduction in number of epidermal cells but not in size under saline conditions. Meiri and Poljakoff-Mayber (1967) reported an increase in epidermal cell number and decrease in epidermal cell size. Both cell size and cell number were reduced in unselected tobacco cell cultures grown in salinized media (Dix and Street, 1975). Peter et al. (1987), working with *Hibiscus cannabinus* reported that the measurements of epidermal cell numbers showed that the salt-induced reduction in leaf area was primarily due to small epidermal cell size. Epidermal cell numbers were also significantly reduced by salinity.

Except at the lower saline level (250 ppm), the palisade cells were larger in size under 500 ppm stress (Plate-6.6A) and had larger diameters under 1000 ppm stress conditions (Plate-6.6B). Under all stress levels the number of spongy cell layers seemed to be increased. David et al. (1979) reported that mesophyll thickness increased with salinity in three species i.e. *P. vulgaris* due to the increase in the length of palisade cells and increased number of spongy cell layers. Diameters

of palisade cells of *Phaseolus vulgaris* and *G. hirsutum* remained constant under saline conditions, but were greater in the *Atriplex* palisade cells. Increase in leaf thickness and the increase in area of mesophyll available for gas exchange have been proposed as mechanisms by which plants must offset the deleterious effects of salinity on cellular photosynthetic capacity (Longstreth and Nobel 1979; Robinson et al. 1983).

As indicated in the results, the chloroplast content in the mesophyll declined slowly for elevated concentrations. At the highest saline level i.e. 1000 ppm (Plate-6.6B) the chloroplast number decreased dramatically. This may be due to the disturbances in the phenomenon of photosynthesis. High concentrations of culture medium *NaCl* generally reduce photosynthesis (Downton 1977; Gale 1975; Neman 1962) although the photosynthetic rates of some species from saline habitat can be rather insensitive (Ackerson 1975; Kleinkopf et al. 1976; Longstreth 1977). Salinity can affect photosynthesis at stomatal or mesophyll levels, depending on type of salinity, duration of treatment, species and plant age (Downton 1977; Gale 1975; Gale et al. 1967; Kleinkopf et al. 1976; Longstreth and Strain 1977; Nieman 1962)

9.13.3 Anatomical changes in root

The results (Plate-6.7-6.8) demonstrate that salinity brought about changes in the root tissues of seedlings subjected to different stress conditions. The salinity apparently increased the size of the cortical cells (Plate-6.7B, 6.8A) and increased the stelar area. Similar changes were observed by Hajibagheri et al. (1985), who recorded 1.5-2 times increase in the stelar diameter and cortical thickness in the roots of *Suaeda maritima* under saline conditions in comparison to the control.

Such changes were also reported by Strogonov (1962) in *Salicornia herbacea* and cotton. The large increase in cortical cell size in the saline conditions is mainly due to increase in vacuole size (Hajibagheri et al. 1985). Contrary to present studies and the results of Hajibagheri et al. (1985), Serrato et al. (1991) reported that seedlings of *P. tamarugo* exhibited smaller root diameters, correlated with differential reductions of the number of the cortex layers and with the reduction in size of the vascular system. Changes in the number and size of epidermal and cortical cells have also been reported by Goss (1977). Strogonov (1964) reported that cell number is little affected by salinity, but cell size increased under saline conditions in halophytes.

The endodermis at lower stress conditions (250, 500 ppm) was undistiguishable and under the highest stress condition (1000 ppm) deteriorated. The deterioration of the endodermis under elevated salinity levels show that high saline stress conditions are harmful for endodermal development (and therefore ion uptake), but Baumeister and Merten (1981) reported that *NaCl* promoted the development of endodermal walls in *Festuca rubra*.

9.14 Fruit-setting, seed-setting and seed-filling in *Arabidopsis thaliana* in response to high metal concentrations

In response to the high heavy metal treatments to the plants, effects on the fruit-setting, seed-setting and seed-fillings were recorded. High concentrations of four heavy metals including $CuSO_4$ (20 ppm), $Al_2(SO_4)_3$ (500 ppm), $ZnSO_4$ (300 ppm) and $CdSO_4$ (200 ppm), as described earlier were selected for study.

The data depicted in Tables-7.1, 7.3, 7.5 and 7.7 show that generally the fruit-setting was affected. For all four heavy metals, the fruit-setting was less affected,

when the maternal parent was pollinated with the pollen donors raised under identical stress conditions (MS-HS x Wild-HS). Exceptionally, in the case of CAD1 (*Cd*-sensitive), the fruit-setting was less affected when the mother parent was pollinated with pollen collected from *Cd* stress-free plants (MS-HS x CAD1-SF). There was no generalized trend in the reductions in the fruit-setting for other cross combinations (MS-HS x wild-SF, MS-SF x wild-HS) in which one of the parents, maternal or paternal, was either stressed or stress-free.

A similar trend was observed for the seed-setting and seed-filling. The reductions were least in the cross combinations, when the donor pollen came from the plants raised under similar heavy metal environmental regimes to those in which the pistillate plants were growing (eg. MS-HS x wild-HS). This trend was similar for the stress conditions of *Cu*, *Al* and *Zn*. In case of *Cd*, as in fruit-setting, the reductions were slightly more in the case of cross combinations where donor and recipient plants were grown under high stress conditions (MS-HS x wild-HS).

Similar conclusions were drawn on the effects of salinity on fruit-setting, seed-setting and seed-filling, discussed already in this chapter. These traits were less affected when pollen donated came from plants under similar saline stress conditions to those in which the maternal plant was growing (eg. MS-HS x wild-HS).

Sacher et al. (1983) recorded more pollen germination of pollen that had undergone gametogenesis in saline-grown plants, on the saline medium than in pollen from non-saline or stress-free plants. From the results he concluded that the greater pollen germination percentage and tube length of saline versus control (non-saline) pollen on saline medium suggested that there may be physiological adaption during pollen development. Approximately 60% of genes expressed in

the gametophyte generation of pollen are also expressed in sporophytic generation of the plant (Tanksley et al. 1981; Sari-Gorla et al. 1986). Therefore, the lesser reductions in the fruit-setting, seed-setting and seed-fillings recorded in these studies in the cross combination (MS-HS x wild-HS) with maternal and paternal plants raised under similar heavy metal regimes may be the result of the same physiological adaption *in vivo* as was suggested by Sacher (1983) for pollen germination and tube growth in saline conditions *in vitro*. Searcy and Macnair (1990) working with *Cu* effects on *Mimulus guttatus* seed production reported that seed/ovule ratio was unaffected, if pollen came from *Cu*-tolerant sources, but was reduced by an average of 24% for *Cu*-supplemented treatments if pollen came from *Cu*-sensitive sources. These workers also reported nonsignificant differences in fertilization percent.

The experiment under discussion suggests that the selection due to environmental factors could occur within the pistil. Under certain environmental conditions, the pistil could be considered as a microcosm reflecting local environmental parameters (Searcy and Macnair 1990). Plants growing on soils rich in heavy metals may contain high concentrations of these metals (Thurman 1981, Baker 1987).

These results suggest that pollen that has undergone development on plants that have been subjected to saline and heavy metal stress has a selective advantage, in that it induces higher levels of seed production in female parents growing in these stress conditions.

The mechanism for this phenomenon is not known, but it would be reasonable to hypothesize that the pollen becomes conditioned to a stress environment during its development in the anthers of stressed plants, and that this preconditioning

allow its pollen tubes to grow more successfully in the styles of female parents growing in similarly stressed environments, where their gynecia may accumulate higher levels of heavy metals or salt.

The environmentally-induced preconditioning of pollen in stressed environments may have important implications for the population genetics of plant populations growing in stressed conditions. This phenomenon would tend to favour fertilization of heavy metal tolerant plants with pollen that had undergone gametogenesis in donor plants that experience similar environmental conditions, leading to inbreeding within heavy metal and saline tolerant populations and, perhaps, long-term genetic isolation and speciation.

the determination of whether these hypotheses are valid requires investigations to demonstrate:

- (a). That the selective fertilization observed in *A. thaliana* is a general phenomenon in stress tolerant plants.
- (b). The mechanism which is responsible for the phenomenon.
- (c). The effect of the phenomenon on inbreeding of stress tolerant and intolerant populations in the field.

9.15 Effect of metals on pollen germination and growth

Four metallic salts including aluminium sulphate, zinc sulphate, iron sulphate and copper sulphate, were selected for these studies to observe their effect on the germination and growth of *Arabidopsis* pollen.

As shown in the results (Table, 7.9, 7.10, 7.11, 7.12) the effect of every element

towards pollen germination and growth was specific. The greatest inhibitory effect was caused by copper sulphate followed by zinc sulphate, aluminium sulphate and iron sulphate.

A few pollen researchers have investigated the effects of heavy metals on certain heavy metal sensitive and tolerant pollen. Searcy and Mulcahy (1985) and Karen and Mulcahy (1985) studied the effects of zinc and copper in *Silene dioica*, *S. alba* and *Mimulus guttatus*. Searcy and Mulcahy (1990) quantified the effects of aluminium toxicity in the pollen and plant of the aluminium tolerant and sensitive lines of tomato (*Lycopersicon esculentum*) Mill.

Brewbaker and Kwack (1964), testing the effect of calcium against pollen growth inhibition in *Crinum asiaticum* showed the following remarkable losses on pollen germination and growth by certain heavy metals. The extent of inhibition by these metals is tabulated as below.

Heavy metal	Concentration (mg/l)	Pollen germ. (%)	Pollen tube length (μm)
Cu	30	3	40
Fe	80	0	0
Co	500	0	0
Mn	2000	0	0
Li	500	0	0
Zn	100	0	0
Ba	500	0	0
Control	-	96	820

The mechanism of inhibition of pollen germination and growth by the heavy

metals still needs to be determined.

9.16 Anatomical changes brought about by heavy metals in the stem leaf and root of *Arabidopsis thaliana*

Four heavy metal salts, $CdSO_4$, $Al_2(SO_4)_3$, $ZnSO_4$ and CuS_4 , at various specific concentrations were selected to test their effects on the stem, leaf and root anatomy. The results are discussed below.

9.16.1 Anatomical changes caused by cadmium sulphate in the stem, leaf and root

Cadmium created enormous changes in the tissues of stem, leaf and root of the plants subjected to this stress conditions. The results obtained during these studies are discussed below.

9.16.1.1 Anatomical changes caused by cadmium in the stem

Very little is known about the effects of cadmium on internal changes in the stem of plants. The results (Plates-8.2-8.5, 8.10-8.13, Table-8.1) obtained during these studies indicated that *Cd* caused substantial changes in the stem tissues of seedlings raised under different *Cd*-stress conditions. The contour of cross-sections was most affected by the stress. As the concentrations were raised the shape of stem become more irregular. This trend was obtained in both genotypes, wild type and CAD1 (*Cd*-sensitive), but CAD1 was more sensitive towards cadmium and was therefore more affected than the wild type. The epidermal cells were generally larger in both treated genotypes than in the control. There were generally more cortical layers with irregular and larger cells in wild type and smaller cortical cells in CAD1 than in the control. In the case of CAD1 large parts of the cross-sectional

area of the stem were disrupted (Plates-8.12-8.13).

The effects of cadmium on the plant shape, epidermis and changes in cortical regions agree with the findings of previous researchers. Petit and Van de Geijn (1978), working with tomato plants, recorded the accumulation of *Cd* in the stem epidermis. It can therefore be postulated that the changes obtained in these studies in the epidermis and cortical area may be by virtue of accumulation of cadmium as stated above. Poljakoff-Mayber (1981) observed the changes in the plastid ultrastructure in the external part of stem cortex, and described these as being similar to those mentioned for osmologically stressed plants, namely the irregular outline of the plants. These changes may be the result of cadmium inducing water stress within the stems. Several authors have found that cadmium toxicity caused water stress by inhibiting water uptake and transport (Lamoreaux and Chaney, 1978; Fuhrer et al. 1981).

In both genotypes the *Cd* affected the stelar region of the stressed plants. In the wild type at 100 ppm (Plate-8.3) the pericycle was disrupted, with irregular cells being present. The phloem, cambium and xylem were more lignified than in control plants. At the 150 ppm stress level, in most of the vascular bundles, phloem and cambium was disrupted (Plate-8.4), the phloem fibres were more densely stained and highly lignified than the control. In the seedlings grown with 200 ppm *Cd*-stress conditions (Plate-8.5), the pericycle were disrupted and nonlignified, but the xylem was more lignified than the control.

Massive tissue disturbance were also recorded in the stem tissues of genotype CAD1. At 100 ppm stress conditions (Plate-8.11), phloem, cambium and xylem were more lignified than in the control. At 150 ppm (Plate-8.12) a large portion of

the stem was destroyed. At the damaged region the phloem fibres were highly lignified and indistinguishable from the cambium cells. The xylem cells were small and lignified. At 200 ppm (Plate-8.13), the vascular system completely disintegrated in highly affected areas. The phloem and xylem cells were lignified, disorganized and indistinguishable.

The changes recorded in the stem tissues in these studies are in the line with the work of certain earlier workers. Serrato et al. (1991), working on the effects of salinity on the seedlings of *Prosopis tamarugo*, reported the disorganization of the vascular bundle of stem. The lignification in phloem fibres, cambium and xylem cells was recorded by several researchers working on salinity, who noted that early lignification is frequently observed in halophytes and nonhalophytes grown in salt (Millner 1934; Saadeddin and Doddema 1986; Solomon et al. 1986).

The *Cd*-stressed plants generally, in both wild type and CAD1 genotypes, showed less chloroplast development than the irrespective controls. Lack of chloroplast in stems of *Cd*-stressed plants confirms the work of Stobart et al. (1985) who mentioned that the long-term exposure of whole plants to cadmium may affect chlorophyll synthesis and thus have an important role in both the chloroplast development in young leaves and the inhibition of photosynthesis.

9.16.1.2 Anatomical changes in leaf

The results recorded indicate that *Cd*-stress created changes in the anatomical structure of leaf of *Arabidopsis*. The *Cd*-stress conditions generally reduced the chloroplast contents in both of the genotypes i.e. wild type and CAD1 (*Cd*-sensitive). The chloroplast content decreased as cadmium concentrations increased. The reductions in chloroplast content of leaf in these studies are in the line with

findings of Bercelo et al. (1988) and Stobart et al. (1985), who reported that cadmium is an important inhibitor of chlorophyll synthesis in new leaves where active chlorophyll production occurs. Long-term exposure of the whole plant to cadmium may affect chlorophyll synthesis and thus have an important role in both chloroplast development in leaves and inhibition of photosynthesis (Stobart et al. 1985).

The results (Plates-8.15 A-B) also indicate that the stomatal number was severely reduced by the cadmium stress conditions, specially in genotype CAD1 which is sensitive towards cadmium effects. Apparently the number of the stomata was reduced with the increasing concentrations of cadmium, on both adaxial and abaxial surfaces of the leaf. The decline in the stomatal numbers in the leaves supports the hypotheses that *Cd*-toxicity induces water-stress by inhibiting water uptake and transport (Lamoreaux and Chaney 1978; Fuhrer et al. 1981). This may in turn be responsible for the reduced leaf area, as recorded by Bercelo et al. (1988) in bean plants. During the present studies the reductions and increments were inconsistent for the recorded leaf thickness (Table-8.2).

9.16.1.3 Anatomical changes in root

Generally, less information is available on the effects of *Cd*-stresses on anatomical changes in the root tissues of plant species. The results recorded (Plates-8.8-8.9, 8.16-8.17, Table-8.3) show that the cadmium stress damaged many parts of root cross-sections and created large changes in the root tissues. In both genotypes, wild type and CAD1, at all stress conditions, the cortical layers were severely damaged and mostly disappeared. Exceptionally, for unknown reasons, in the case of 100 ppm *Cd*-stress, the cortex seemed to be intact in genotype CAD1 (Plate-

8.16B). The deterioration of cortical cells is one of the early effects of cadmium in roots, which takes place due to the inhibition of cortical cell division (Vazquez et al. 1992).

The endodermis was generally damaged in both genotypes under every stress condition. In the case of the wild type, the pericycle, phloem and cambium were undistinguishable and more stained. The presence of undifferentiated vascular bundles is in line with findings of Vazquez et al. (1992) who found similar results when working with *Phaseolus vulgaris*. In the case of CAD1, the pericycle cells differentiated from phloem cells in the roots raised under 150 and 200 ppm of *Cd*-stresses, but unexpectedly in the genotype CAD1, in the roots treated with 100 ppm, the pericycle and phloem were indistinguishable, densely stained and highly lignified. The xylem cells were larger than the control in both genotypes under stress conditions. The stress conditions increased the stelar diameter. The high lignification of pericycle, phloem and cambium may be due to the accumulation of phenolic compounds. The accumulation of phenolic compounds in plants stressed by low temperature, aerobic conditions and disease suggest that the symptom probably is a general response to stress. (Hecht-Buchholz et al. 1987).

Reviewing the literature, it can be quoted that Malone et al. (1978), studying the effect of *Cd* on corn roots, observed that small concentrations of *Cd* (100 µg/l) reduced the number of lateral roots formed by *Zea mays* plants. It was therefore suggested that the general reduction of root growth reported (Hassett 1978) might be the result of a reduction of cell division.

Contrary to expectations, histological structure of roots in the wild type was more adversely affected at every cadmium concentration than that of CAD1 (*Cd*-

sensitive). This may be due to the reason that wild type plants were allowed to grow for five days under stress conditions, whereas CAD1 plants were stressed for two days only. As mentioned in the methods, this was due to the sensitive nature of CAD1 plants could not withstand *Cd* treatment for more than two days.

9.16.2 Anatomical changes caused by aluminium sulphate in the stem, leaf and root

Aluminium caused distinct changes in the tissues of stem, leaf and root of *A. thaliana*. The results recorded are discussed below.

9.16.2.1 Anatomical changes in stem

The results indicated in Plates-8.19-8.21 (Table-8.1) show that treatment of $Al_2(SO_4)_3$, brought changes in the stem tissues. At 200 ppm $Al_2(SO_4)_3$, the cortical cells were irregular and larger in size and shape, with very few chloroplasts. The pericycle was lignified continuously, as were xylem cells. In the vascular system the phloem fibres, cambium and xylem were more lignified than the control. The cambium layers were disturbed in certain vascular bundles. There were more cell layers in the cortical area, and the cortex contained more chloroplasts. In the vascular system, phloem fibres, cambium and xylem were all lignified.

Very little is known regarding the changes caused by *Al* in stem tissues. As already discussed, the changes in the epidermal region caused by cadmium are the result of its accumulation in the epidermal cells (Petit and Van de Geijn 1978). The changes in the cortical region affected by *Al*-treatment may be similar to those described for *Cd* by Poljakoff-Mayber (1981). The accumulation of chloroplasts in the cortical region of aluminium-stressed stems seems to be a similar effect to that found in *Al*-stressed leaves (see below) having high chloroplast numbers

at elevated *Al* levels. These depositions of chloroplasts seems to be in line with findings of Samarkoon and Rauser (1979), who reported that certain heavy metals like nickel and cobalt induced starch accumulation. From present studies it could be hypothesised that photosynthesis may be enhanced during *Al*-stress conditions.

The causes of the changes in the vascular system by *Al*-stress are not known, but Serrato et al. (1991), working on the salinity effects on anatomy of *Prosopis tamarugo* reported many similar changes in the vascular system of the stem.

9.16.2.2 Anatomical changes in leaf

The results (Plate-8.22, Table-8.2) for plants raised under *Al*-stress conditions show that, apparently, there was no reduction in the number of stomata. The epidermal cells were stimulated and became slightly larger at lower concentrations, but were adversely affected at the highest (500 ppm) concentration (Plate-8.22C). Generally a decrease in palisade parenchyma and spongy mesophyll cells was observed. The intercellular spaces were also reduced at increasing levels of stress. The chloroplast number seemed to increase with increasing concentrations of aluminium. The deposition of more chloroplasts in these plants, as already mentioned, may be in accordance with the findings of Samarkoon and Rauser (1979), who reported that nickel, cobalt and copper induced starch accumulation, by inhibition of vein loading.

9.16.2.3 Anatomical changes in root

Little is known about cellular changes occurring in *Al*-stressed roots. The results obtained (Plates-8.23, Table-8.3) indicate that at lower concentrations i.e. at 100 ppm, the cortical cells were less affected, but elevated levels damaged the

entire cortical region. The deleterious effects on cortical cells are in accordance with the findings of McQuattie and Schier (1990) who worked with red spruce *Picea rubens* seedlings. The literature indicates that aluminium induced loss of cells from peripheral layers and the formation of intercellular spaces in the cortex of red spruce *Picea rubens* root is probably due to the breakdown of the middle lamella, which consists mainly of calcium pectate (Poovaiah 1988). Since calcium uptake is reduced by *Al*, this causes cell separation because of calcium deficiency (Schier 1985) and dissolution of calcium pectate in the intercellular walls.

Relating the effects of *Al* on root with the results already discussed for the effects of *Cd*-stresses on root, it would be noted that both of these metals have perhaps similar effects on the cortical region of root. Similarly Hecht-Buchholz (1979) working with *Cd*-effects on potato and McQuattie and Schier (1990) working with the *Al*-effects on red Spruce *Picea rubens* seedlings, found comparable effects of *Cd* & *Al* respectively on both plant species.

There is evidence that the *Al* effect on plants depends not only on the absolute concentration of *Al*, but also on the relative concentration of *Ca*. Root damage has been observed when Norway Spruce (*Picea abies*) seedlings were grown in nutrient solution with low calcium/aluminium ratios (Jorns and Hecht-Buchholz 1985; Rost-Siebert 1983).

In the present experiments, at lower concentrations, the endodermis (Plate-8.23 A,B) was found intact but at higher levels (Plate-8.23C) the endodermis was completely destroyed. Pericycle cells were larger and more lignified than the control. Phloem was lignified. Xylem cells were comparatively large for the elevated stress conditions.

The lignification in the pericycle and vascular system may be due to the accumulation of phenolic compounds. The results for the lignification obtained in the stelar region are in line with the work of earlier researchers, who described that the accumulation of phenolic compounds in roots of seedlings is commonly observed in response to aluminium stress (Hecht-Buchholz 1987; Jorns and Hech-Buchholz 1985; Metzler and Oberwinkler 1986).

9.16.3 Anatomical changes caused by zinc sulphate in the stem, leaf and root

Zinc-stress brought changes in the anatomy of stem, leaf and root of *A. thaliana*. The details of the results recorded are discussed below.

9.16.3.1 Anatomical changes caused by zinc in the stem

Very little is known regarding the changes caused by the *Zn*-stress conditions in the stem of plant species. The changes recorded in the stem tissues, by virtue of *Zn* stress conditions, are discussed below.

Zinc-stress conditions created changes in the tissues of the stem (Plate-8.24-8.26, Table-8.1). Generally, epidermal and cortex cells were larger than control cells. The chloroplast content varied for different stress treatments. Groups of unusual lignified cells (Plate-8.25B) appeared in cortex. The stele, the pericycle, phloem, cambium and xylem were more lignified than the control.

The changes in the epidermis and cortical layers observed under *Zn*-stress conditions, seem to be similar to those recorded for cadmium and aluminium during these studies. The lignified condition of the stelar region is in accordance with the findings of previous workers like Rauser (1973) and Robb et al. (1980), working on

bean plants, who mentioned that zinc treatment in plants enhanced the synthesis of phenolic compounds. The results for increasing lignification in pericycle and xylem vessels due to the enhancement of synthesis of phenolic compounds for applied *Zn* were confirmed by Paivoke (1983), while working on pea (*Pisum sativum*).

9.16.3.2 Anatomical changes caused by zinc in the leaf

In the case of plants studied for the effects of *Zn*-stresses on the leaf anatomy (Plate-8.27, Table-8.2), the results show that under all stress conditions the stomatal number was found to be less on both sides of leaves from plants grown under 100 ppm *Zn*-stress than in the control plants. The stomatal aperture visually seemed to be bigger under elevated stresses. In all of the stressed leaves the chloroplast content seemed to be greater than the control. The stress conditioned leaves also contained many intercellular spaces. The high frequency of chloroplasts and intercellular spaces, could be the result of larger stomatal size; resulting in more air exchange with enhanced photosynthetic activities. These results may be consistent with the findings of Samarakoon and Rauser (1979), who reported starch accumulation in the leaves stressed with copper, cobalt and nickel, as already discussed in this section.

The palisade parenchyma cells were scattered and larger in size than in control plants and differed in shape for different stress conditions. The increase in cell size of palisade parenchyma and spongy mesophyll cells may in turn result in greater leaf thickness as generally observed under lower zinc concentrations (Table-8.2). There appears to be no literature on these phenomena.

9.16.3.3 Anatomical changes caused by zinc in the root

The results obtained (Plate-8.28, Table-8.3) for the roots treated with $ZnSO_4$ indicate that, like the control, the cortex layers were intact in stressed seedling roots. These were only partially damaged under the highest Zn -stressed conditions of 300 ppm (Plate-8.28C). In general the cortical cell size was reduced. The endodermis at lower levels of zinc was unaffected, but at higher concentrations its arrangement was disturbed. Size of the stele diameter and xylem cells decreased, compared with the control.

The partial losses of cortical cells in these studies are in the agreement with the work of Paivoke (1983), who worked with *Pisum sativum* and recorded the loss or damage of cortical tissue when seedlings were grown under 0.1 mM and 1.0 mM zinc.

The extent of lignification in the pericycle and vascular area differed from the results found with other metals, since the level of lignification in stressed roots was similar to the control (Plate-8.8A). But Paivoke (1983), working with pea seedlings at even lower concentrations of zinc than were used in these studies, recorded a high degree of lignification. It is quite possible that this difference may be due to the different plant species used for respective studies. But it is a general observation drawn from previous studies, that certain anatomical studies suggested that zinc treatment enhances the synthesis of phenolic compounds in plants (Rauser 1973; Robb et al. 1980).

9.16.4 Anatomical changes caused by copper sulphate in the stem, leaf and root

The treatments of $CuSO_4$ brought internal changes in the tissues of the stem, leaf and root of *A. thaliana* plants. The results recorded are discussed as below.

9.16.4.1 Anatomical changes in stem

The results (Plates-8.29-8.31, Table-8.1) show that $CuSO_4$ caused large scale disturbances in the histological development of stem, stressed with various concentrations of the metal. Under the highest stress condition the epidermal cells were larger than the control. The cortical cells became larger than the control under heightened concentrations of $CuSO_4$. The cortex lost chloroplasts under every stress condition. The higher the stress, the more the pericycle deteriorated (Plate-8.30B, 8.31B). The lignification in the phloem fibers, cambium and xylem increased with increasing concentration of $CuSO_4$.

The decrease in the chloroplast content of cortical cells is in agreement with the work of Stobart et al. (1985), as already described, and results recorded during these studies on cadmium stress conditions. The changes in the vascular bundles are in accordance with the work of Serrato et al. (1991) and results obtained for cadmium, aluminium and zinc stresses in these studies.

9.16.4.2 Anatomical changes in leaf

The results obtained for the plants treated under $CuSO_4$ stresses (Plate-8.32, Table-8.2) showed larger epidermal cells on adaxial and abaxial surfaces. There were larger palisade parenchyma cells with different cell shapes for different stress concentrations. Chloroplast number was very high in comparison with the control for the leaf treated with the 10 ppm (Plate-8.32A) Cu -stress condition. The chloroplast number declined drastically at higher stress levels. The presence of

many chloroplasts confirms the results of Samarakoon and Rauser (1979), as already discussed. The discovery of reductions in chloroplast content at elevated copper concentrations conflicts with the observations of these earlier workers. It is quite possible that at certain levels of copper chloroplast accumulation may be taking place, but there is no literature available on this.

9.16.4.3 Anatomical changes in root

A little is known about the anatomical changes created by *Cu*-stress conditions in the roots of plant species. The results (Plate-8.33, Table-8.3) indicate that *Cu*-toxicity damaged the cortical area of the root. With increasing doses the cortex layers were more damaged. The deterioration of cortical parenchyma layers was similar to that recorded during these studies for *Cd* & *Al*. These results also confirm the findings of Paivoke (1983), who obtained similar results while observing the *Zn*-effects on the anatomy of pea seedlings.

Unlike the control the endodermis in *Cu*-treated roots was destroyed and undiscernible. Similar results were recorded during these studies for *Al*, *Cd* & *Zn*. These results agree with findings of Paivoke (1983).

The pericycle, phloem and cambium were indistinguishable and lignified. Similar lignification in the stelar area of roots treated with *Cd* & *Al* was observed during these studies, whereas in the *Zn*-treated roots the extent of lignification was less. Lignification takes place due to the enhancement of synthesis of phenolic compounds, which is stimulated by certain heavy metals (Paivoke 1983; Rauser 1973; Robb et al. 1980) or as stated earlier, certain environmental stresses like temperature, aerobic conditions and diseases (Hecht-Buchholz et al. 1987)

Under all heavy metal stress conditions applied to the roots, except *Zn*-stressed roots, the xylem cells of treated roots were bigger than in the control.

Comparing the equivalent concentration (200 ppm) used for different heavy metals in experiments the following changes were observed in the stem, leaf & root tissues/cells.

The data revealed in Table-8.1 that the *Cd* & *Zn*-stresses increased the stem diameter (SD) in wild type (WT) genotype to varying degrees, with the increase in the stem diameter being greater in *Zn*-treated plants (20.0%) than in cadmium treated plants (9.71%). Conversely both aluminium (WT) and cadmium (CAD1) decreased SD, with a greater reductions seen in the *Cd* (CAD1) treatments in comparison to the controls. In the case of the epidermis cell size cadmium & aluminium treatments reduced the cell size of WT-plants. The epidermis cell size was reduced slightly by cadmium (1.37%) and substantially by *Al*-stresses (8.22%) in wild type. The epidermal cell size was slightly stimulated by cadmium (1.39%) in CAD1 and sufficiently by the *Zn*-stress (27.39%) in WT-genotype. The cortex cell size was adversely affected by the cadmium (44.44%) in CAD1 but was highly stimulated by zinc (141%), aluminium (56.25%) and cadmium (53.75%) respectively in WT against the controls. The cross sectional diameter of the vascular bundle (CSDVB) was increased by the stresses of all heavy metals in the WT, except that it was decreased by the cadmium treatment in CAD1. The pith cell size was increased by the treatments of cadmium, aluminium in the WT and was reduced by the cadmium in CAD1.

Looking to the changes caused by the equivalent concentration (200 ppm) of heavy metals in the stem, it could be noted that generally all of heavy metals stim-

ulated the size of tissue/cell in the WT-genotype & reduced it in CAD1, which is cadmium sensitive. This may be due to the degree of tolerance against heavy metals between both genotypes (WT, CAD1). The results also indicate the possibility that the CAD1 could be sensitive to other heavy metals also, if this is so, then it can be postulated that all heavy metals may have similar mechanism to affect plant tissues, only with different extents of phytotoxicity.

Concluding the effects of similar stress level (200 ppm) of heavy metals on the leaf tissue/cell, it was observed that the leaf thickness was increased under stress regimes of cadmium and zinc & decreased under *Al*-stress, compared with the control. The palisade cell size was greatly increased by the zinc stress (83.78%), and by aluminium & cadmium (CAD1), but its cell size was reduced in the WT under cadmium stress conditions. The size of the intercellular spaces were decreased under heavy metal treatments in the WT, but it increased in genotype CAD1.

The comparative results (Table-8.3) for a similar stress concentration (200 ppm) indicated that the stele diameter of the root was increased under cadmium stress in both genotypes WT & CAD1, while it was reduced in comparison to the control in aluminium and zinc treatments. The xylem cell size was increased generally under most heavy metal stress regimes, but was reduced under zinc stress in comparison to the control. The highest stimulation in the xylem cell size was observed under *Cd*-stress (111.07%). The cortex was generally disrupted under all heavy metal stresses, but it was only found intact under zinc stress condition, where it reduced by 31.96% relative to the control. The intact nature of the cortex only under zinc-stress perhaps supports the idea that every heavy metal has certain specific limits of toxicity against plant tissues.

Overall the results conclude that the mechanism of toxic effects to the histological structure of the plant tissue/cell could be similar for most of the heavy metals, but their extent of phytotoxicity may vary and could not be generalized for all metals. The presence of heavy metals in the wastes from polluting sources has recently become an increasing problem (Cunningham et al. 1975; Duda & Pawiuk 1975). The toxic effects of heavy metals on plant and losses in productivity may be classified a major problem. Knowledge of toxic effects of heavy metals on the plant anatomy may contribute to the general understanding of the primary toxicity mechanisms of metals and of the tolerance phenomena in living organisms. An alternative strategy to overcome heavy metal toxicity problems is to breed heavy metal ecotypes by genetic understanding the mechanisms underlying basic anatomical changes in the plant tissues.

Due to the late availability of CAD1 (cadmium sensitive) line, the effects of cadmium could not be studied on its gametophyte. It would be very interesting to investigate, how do the *Cd*-stress regimes are influencing its pollen parameters in comparison with wild type.

In theory pollen of wild type may tolerate more doses of cadmium for its pollen germination and pollen growth than CAD1. If it works so, it would lead to hypothesise in two ways.

(a). As the sporophyte of CAD1 already proved to be sensitive to cadmium, the present result will support the concept of haplo-diploid gene-overlap.

(b). It would support the idea that selection for stress factors takes place in anthers of plant during gametogenesis.

9.17 Future research

The research work reported in this thesis, provides the substantial insight into the effects of certain environmental pollutants on the sporophytic and gametophytic generations of the model plant (*Arabidopsis thaliana*). Further work is now required to determine whether the results reported here are generally applicable to all members of the *Cruciferae*, all dicots and all angiosperms. It is now an established fact that the extensive, haplo-diploid overlap in gene expression and the special features of the male gametophyte generation make pollen an important tool for practical applications in the plant research. Correlations and simultaneous changes between sporophytic traits and gametophyte have been also recorded during these studies.

The knowledge acquired could be exploited beneficially for practical plant improvement against stress conditions, specifically for the following purposes:

1. Screening of crop plants against salinity.
2. Selection of heavy metal tolerant plants.

For serious screening of crop plants, still an important step is missing in the present studies. That missing step is a "**Pollen Rescue Technique (PRT)**" which could not be developed, because of time limitations. The PRT will enable the researcher to germinate the pollen *in vitro* under semi-lethal levels of any of the stress regimes and pollinate/fertilize the pistil *in vivo* with stress-surviving pollen by the technique developed.

Further work needs to be carried out to determine whether the same methodology could be used for:

1. Screening against pesticides, herbicides & fungicides.
2. Selecting against chilling and high temperatures regimes.
3. Screening against water-stresses or drought conditions.

Chapter X

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