Research Journal of Environmental Sciences

ISSN 1819-3412

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Environmental Sciences 2 (6): 415-423, 2008
ISSN 1819-3412

Influence of Salinity at Early Stage of Flowering on the Development of Male Gametophyte in Canola (*Brassica napus* L.) cv. Symbol

[1]Homa Mahmoodzadeh and [2]Maasoumeh Bemani
[1]Department of Biology, Faculty of Science, Islamic Azad University of Mashhad, Iran
[2]Department of English, Islamic Azad University of Mashhad, Iran

Abstract: Effects of salt stress on developmental stages of male gametophyte were investigated, using both light and electron microscopy through the popular cyto-histological methods. The findings were then compared with those obtained from the unstressed plants. It was found out that salt stress could cause significant changes on the general growth of plants as well as a reduction of the number of pollen grains in anthers at the beginning of flowering stage. In spite of the normal appearance of the florets, the growth of anthers was abnormal, appearing to be shriveled, colorless and small. The other effects observed during various stages of development are as follow: pre-time destruction of the anther wall, degeneration and abnormal forms of pollen grains. As a result, such reduction of the number of pollens and their abnormality of the form and structure could ultimately lead to decrease of crops yield.

Key words: Canola, salt stress, male gametophyte

INTRODUCTION

As a result of abiotic stresses, crop yields can be 4 to 20-fold less than yields under optimal growth conditions (Boyer, 1982). These abiotic stresses include water availability, temperature extremes, nutrient levels, fluence rate, soil salinity and pollution. Among stresses, soil salinity is an ancient problem that had existed long before the advent of agriculture. Today, about 20% of the cultivated land on Earth is affected by ion accumulation (Zhu, 2002). High salinity has two major deleterious effects on plants: the first is caused by water deficit, which results from increased solute concentration or osmoticum. the other is ion toxicity which inhibits enzymatic function of key biological processes (Zhang and Blumwald, 2001). In response to these deleterious effects, plants have evolved multiple mechanisms to overcome environmental stresses. One of these mechanisms, a decrease in plant fecundity, involves aborting ovules and/or pollen, then shunting resources from reproductive activities into metabolic reactions that increase stress tolerance. Salt stress also can induce or accelerate senescence of the reproductive organs. For example, in a study by Asch and Wopereis (2001), salinity reduced the yield of rice approximately 45% as a result of spikelet sterility and, in surviving seed, a reduction in seed weight. In field-grown cotton, salt stress was a major cause of seed abortion, reducing both yield and quality (Davidonis *et al.*, 2000). The research studies carried out by Namuco and O'Toole (1989) and Westgate and Peterson (2004) showed that the developing microspores were very sensitive to salt stress. Also, Sun *et al.* (2004) found out that the in Arabidopsis microsporocytes, grown in salty conditions, did not mature into viable pollen grains. instead, they became vacuolated and majority of them senesced within two days, leaving anthers filled with pollen corpses. Interestingly, mature pollen was unaffected by even very long periods of salt stress; thus the extent to which pollen was affected by stress depended upon the developmental stage of the stamen.

Corresponding Author: Homa Mahmoodzadeh, Department of Biology, Islamic Azad University of Mashhad, Iran

In the recent years, Canola has been used as a proper alternative to oil seeds and its new varieties have been reformed and widely cultivated because of its high rate of oil as compared to the other oil seeds, its effective role in apiculture industry, its being apt to be cultivated for a second time and in autumn, as well as its need for water on a minimum scale. The present study investigates the developmental stages of the male gametophyte in canola as well as the effects of salt stress on these stages.

MATERIALS AND METHODS

The experiment was conducted in Faculty of Science, Islamic Azad University of Mashhad, Iran (2006).

The seeds of canola (cv. Symbol) were obtained from the Research Center of Agriculture and Natural Resources in Khorasan. The processes of culturing and treatment took place in a green house where the experiments were carried out on 5 groups, one of which the control received no NaCl, while the other 4 groups were treated with 3, 6, 9 and 12 dS m^{-1} NaCl solution. At the beginning stage of flowering, the florets (1-3 cm) were sampled out from both the control and treatment groups. Then, to be prepared for microtome sections, the samples were fixed, using Formaldehyde Acetic Acid (FAA) for 12-14 h. Next, they were dehydrated, using the solutions of pure ethanol, butanol (1:1) with the percentages of 50 to 100. Having been paraffinized and moulded, both the latitudinal and longitudinal sections of 12 μ were then prepared with microtome. Later, they were dyed with Hematoxylin-eosin. Photo microscope Olympus BH-2 was used for photography and microscopic investigations. To study the pollens, the pollen grains were first taken out of the anthers and then, having been fixed on stub. they were coated with an alloy of gold-palladium to be studies with SEM microscope of the model LEO 1450 VP.

In order for the pollen grains in each anther to be counted, the mature anthers from five different flowers of five plants were collected. Each one of the anthers was then placed in a droplet of water on a glass lam to be ripped up with a needle. The released grains in the droplet of water were then counted (Bennet *et al*., 1973).

RESULTS

The developmental stages of the male gametophytes were determined through a study on the longitudinal sections of anthers in the control plants. In the wall structure of the young anther, where the spore-producing cells surround the mother cell, the following layers are identifiable, respectively from the outer layers to the inner ones: epidermis, mechanical layer, middle layer(transition cells), nutritious layer (tapetum) (Fig. 1). Following the development of the pollens, the pollen mother cells go through meiosis division, during which the unique wall of callose is formed around the pollen mother cells (Fig. 2). As a result of their meiosis, the tetrads are created (Fig. 3), around each of which a special callose wall is constructed. Then, the tetrads separate from each other where each one of the meiospores is turned into a young microspore. During this stage, some of the cells from the nutritious (tapitum) layer have already been exhausted and displaced (Fig. 4). Next, the microspores rapidly go through their mitosis division. The exine can be identified on the surface of each microspore where first the young pollens and then the mature and dinuclear one are formed (Fig. 5). The mature pollens are oval with two cells: vegetative (the bigger one) and generative (the smaller one) (Fig. 7).

Meanwhile, some of the cells from the nutritious layer of the anther wall are released, go into the pollen sac and approach the developing pollens where they are lysed and their remaining substances supply food for the pollen grains (topiplasmedy). At this stage, the anther wall has already been

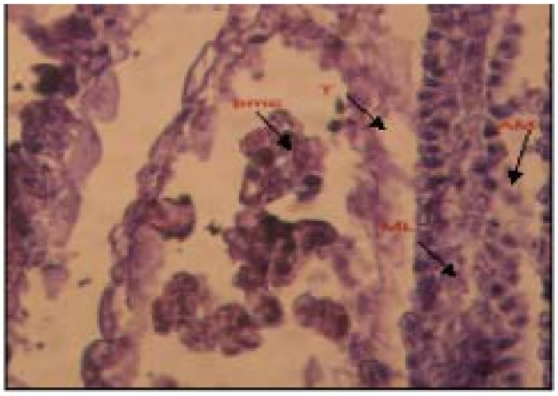

Fig. 1: Longitudinal-section of young anther (LS). Hematoxylin-eosine staining (×40). Pmc-pollen mother cells; ML-middle layer; AM-mechanical layer; T-tapetum

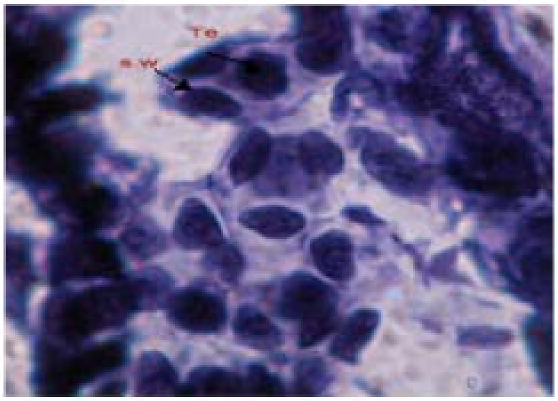

Fig. 2: Callose special wall surrounded tetrads staining Hematoxylin-eosin (× 100). Te-tetrad; SW- callose special wall

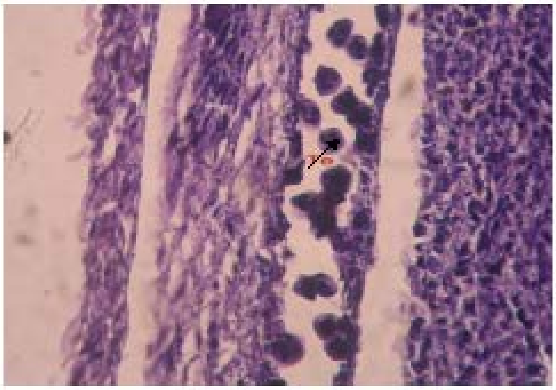

Fig. 3: Tetrad formation in the young anther staining Hematoxylin-eosin (×40). Te-tetrad

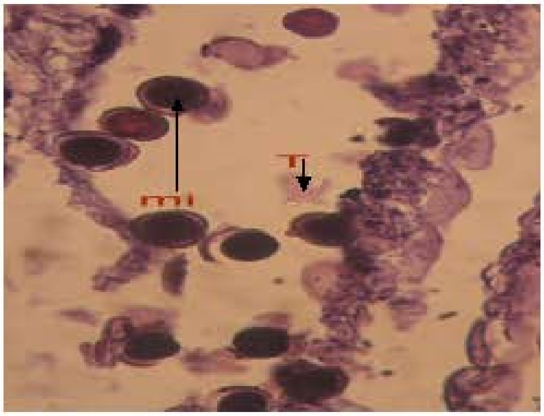

Fig. 4: Young anther staining Hematoxylin-eosin (×100). T-tapetal layer cells; mi-microspore

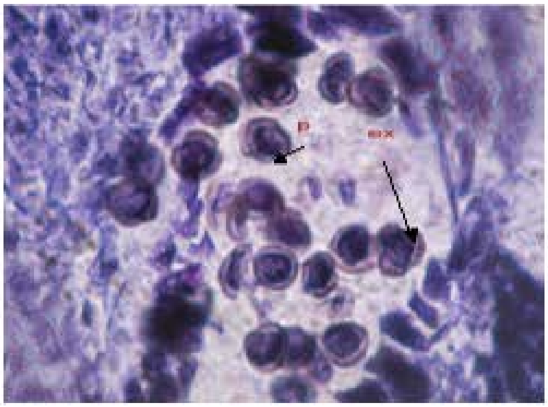

Fig. 5: Longitudinal-section of anther Hematoxylin-eosin staining (×40).p-young pollen; ex-exine

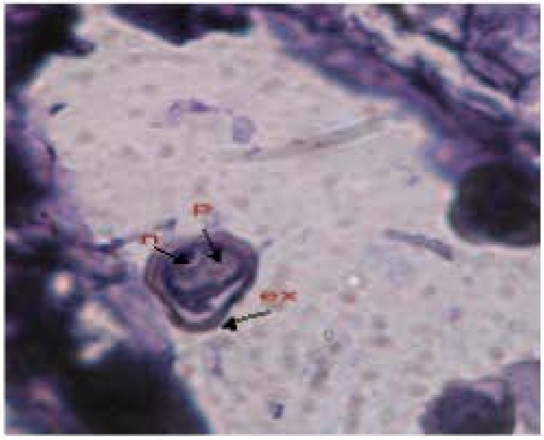

Fig. 6: Longitudinal-section of anther Hematoxylin-eosin staining (×100). p-pollen grain; n-nucleus; ex-exine

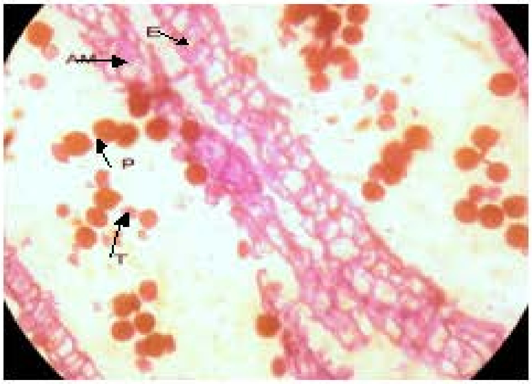

Fig. 7: Longitudinal-section of mature anther Hematoxylin-eosin staining (×100). E-epidermis; AM-mechanical layer; T-tapetal layer cells; P-pollen grain

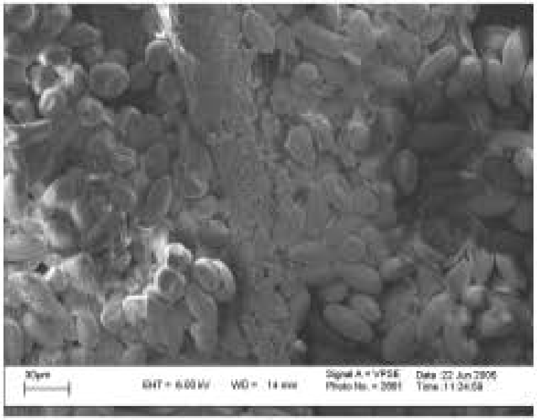

Fig. 8: Scanning electron microscopy images of pollen grains in the anther

reduced to only mechanical layer and epidermis. Finally, the anther splits and releases the pollens. Using SEM microscope, the pollen grains were found to be oval and exine with reticulated ornamentation and triporate (Fig. 8, 9).

The comparative studies on both the control group and the treatments showed that both general and morphological structures of canola undergo some changes as affected by salt (Fig. 10). In the treatments of higher concentration of salt solution (12 dS m^{-1}), the plant started flowering later than those of the control group while for the treatment of lower concentration (3 dS m^{-1}), the durations of growth and development do not last as long and the plants are prepare to go up to the stage of generation as a result of which they start flowering earlier (Majd and Jafari, 1996).

In the control plants, microsporocytes have one single nucleus each which appear quite condensed through histochemical methods of staining (Fig. 6), indicating their rapid growth and development. As for the plants treated with salt, in spite of the normal appearance of the florets, the anthers did not grow normally and some of them were shriveled, colorless and small. Also, the anthers full of pollen grains were deformed and deteriorated (Fig. 11). In the treated samples, the anther walls were destroyed faster than those of the control ones so much so that their flowers were of 2 cm long as opposed to the flowers of the control group with a length of 3 cm.

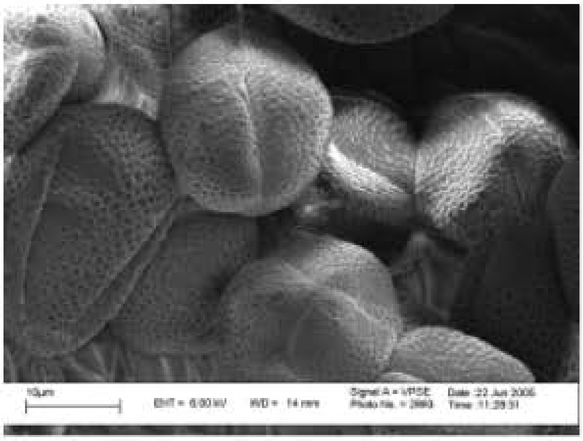

Fig. 9: Scanning electron microscopy images of pollen grains with reticulated ornamentation and triporate

Fig. 10: Canola plants in greenhouse (a) control; (b) 12 dS m^{-1} treatment

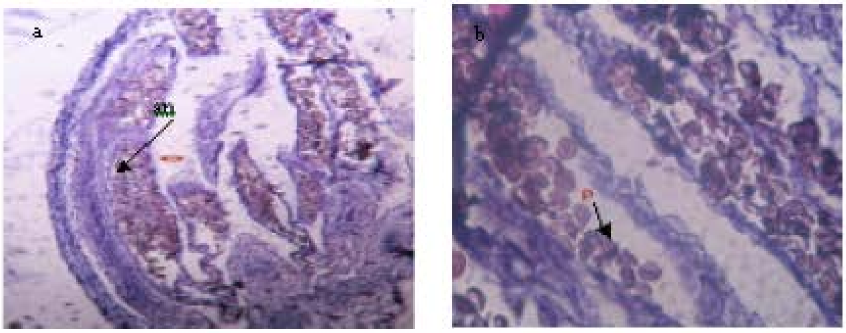

Fig. 11: Longitudinal-section of growing flower of 12 dS m^{-1} treatment Hematoxylin-eosin staining (a) anthers in growing flower (x20) (b) arrow indicate collapsed pollen grains (x40) an-anther; p-pollen grain

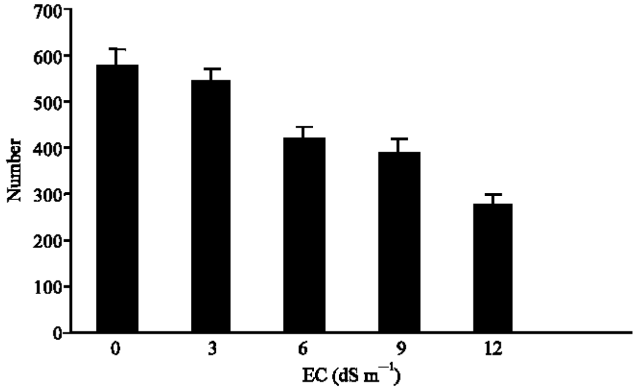

Fig. 12: Effects of salt stress on number of pollen grains per anther

There was a significant reduction of 52% on the number of pollen grains in the group of treated plants (12 dS m^{-1}) as compared to the control group (Fig. 12).

The growth of filaments causes the stamens to brush against the stigma and coat it with pollen. In control plants, stamen filaments undergo rapid growth. In response to salt stress, however, there was a decrease in the growth rate of stamen filaments. While filaments from stressed plants grew more slower than the control groups, they did manage to pollinate the stigma. This slower growth delayed pollination by 14 to 24 h, although this delay did not prevent fertilization of female gametophyte.

DISCUSSION

As ovules develop into seeds, the associated cells and tissues go through a number of intricate developmental and physiological processes. Since plants need a lot of food supply for reproduction, they regulate the development of their pollen grains, ovule and seeds as a reaction to the changes in the environment. Under some crucial circumstances, the developing pollen abort in many seed-bearing plants.

Using the usual histochemical methods, the present research has investigated the developmental stages of the male gametophyte in canola with a respect to the conditions of salt stress. The findings about the inhibitoring effects of salt stress on the growth of male gametophyte are in accordance with those of the past research (Moss and Downy, 1971; Saini and Aspinall, 1981; O'Toole and Moya, 1981; Westgate and Boyer, 1986; Sheoran and Saini, 1996).

As shown by Namuco and O'Toole (1986) in their research, the growing microspores are very sensitive to salt stress. In some of the crops such as bean, canola, corn and soybean, the stress conditions cause death for the mature gametophytes (Moss and Downy, 1971; Sage and Webster, 1990; Kokubun *et al*., 2001; Young *et al*., 2004). Thus, the crucial conditions of the environment may lead to the stop or failure in the process of a natural growth for the male gametophyte and the developing pollen grains; however, such failures at the developmental stages depend on the process at which the plant undergoes the stress.

Development of male gametophyte in plants is highly sensitive to salt and water deficit stress during meiosis in the microspore mother cells. At this stage, the deficiency of water prevents further development of microspores or pollen grains and causing male sterility. Such defects do not seem to be caused by direct effects on reproduction tissues, but as an indirect effect of dehydration on the growing organs, like leaves.

The mechanism underlying this stress response involves probably a long-distance signaling molecule in the organs exposed to the stress which, in return, affects the fertility in the reproductive tissues. In this regard, many researches show the role of abscisic acid; however, the most recent evidence tends to reject a role for this hormone in the induction of male sterility (Morgan, 1980; McRae, 1985). Failure of male gametophyte development caused by the stress conditions is due to some disturbance in carbohydrate metabolism and distribution within anthers as well as the adverse effects of key sugar-cleaving enzyme, acid invertase. Since invertase gene expression can be regulated by the concentration of sugar, it is possible that reduction sugar delivery to reproductive tissue upon inhibition of photosynthesis by stress is the signal that triggers metabolic lesions leading to failure of male gametophyte development (Saini *et al*., 1984; Dorion *et al*., 1996).

The arrest of male gametophyte development, leading to pollen sterility is very common for most of the cereals. The drought caused by salt stress during the period from stamen initiation to anthesis causes serious yield reduction in many cereal and dicot crops (Sato, 1954; Wells and Dubetz, 1966; Dubetz and Bole, 1973; Fischer, 1973).

Saini *et al*. (1984) and Lalonde *et al*. (1997) have also studied developmental abnormality in anthers upon exposed to salt stress and water deficit during meiosis in wheat. As shown in their studies, microspore mother cells apparently complete meiosis, but further microspore development is arrested at various stages. The most common sign of developmental failure is the of displacement of microspores from their normal peripheral position. Such a change could occur at anytime, between the young microspore stage and the first pollen grain mitosis; the timing apparently depends on the cultivar. In some anthers, the abnormal vacuolation of the topetum can be seen soon after meiosis. thus, it is possible that tapetal dysfunction leads to the loss of microspore orientation.

The researches carried out by Saini and Aspinall (1981) and those by Sheoran and Saini (1996) have demonstrated that the disoriented pollen grains have delute cytoplasm, little or no intine but normal exine and they fail to accumulate starch, which is a major constituent of fertile grass pollen.

As found out by Skazkin and Zavadskaya (1957), some abnormalities in chromosomal pairing and separation during meiosis in pollen mother cells of rather severely stressed barley, are the male sterilant effect of water stress. Also, Namuco and O'Toole (1986) reported that a number of meiotic abnormalities in ware-stressed rice started to increase at relatively moderate stress.

As a whole, present findings show that salt stress conditions adversely affect the general development of canola, causing a reduction of the number of pollen grains in the anthers at the early stages of flowering. Furthermore, it was found out that the salt stress not only causes the unusual growth of anthers, but also affects their appearance like being shriveled, colorlessness and small size. Among the other findings are: early destruction of the anther wall, exhaustion of the pollen grains and deformity of the pollens which all prove that salt stress results in a reduction of crops through adverse effects on the development of male gametophytes in canola.

REFERENCES

Asch, F. and M.C.S. Wopereis, 2001. Responses of field-grown irrigated rice cultivars to varying levels of flood water salinity in a semi-arid environment. Field Crops Res., 70: 127-137.

Bennett, M.D., M.K. Rao, J.B. Smith and M.W. Bayliss, 1973. Cell development in the anther, the ovule and the young seed of *Triticum aestivum* L. var. Chinese spring. Philos. Trans. R. Soc. Lond. B., 266: 39-81.

Boyer, J.S., 1982. Plant productivity and environment. Science, 218: 443-448.

Davidonis, G.H., A. Johnson and J.A. Landivar, 2000. Cotton mote frequency under rainfed and irrigated condition. J. Cotton Sci., 4: 1-9.

Dorion, S., S., Lalonde and H.S. Saini, 1996. Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrates metabolism in anthers. Plant Physiol., 111: 137-145.

Dubetz, S. and J.B. Bole, 1973. Effect of moisture stress at early heading and of nitrogen fertilizer on three spring wheat cultivars. Can. J. Plant Sci., 53: 1-5.

Fischer, R.A., 1973. The Effect of Water Stress at Various Stage of Development on Yield Processes in Wheat. In: Plant Response to Climatic Factors. Slatyer, R.O. (Ed.). UNESCO, Paris, pp: 233-241.

Kokubun, M., S. Shimada and M. Takahashi, 2001. Flower abortion caused by preanthesis water deficit is not attributed to impairment of pollen in soybean. Crop Sci., 41: 1517-1521.

Lalonde, S., D. Beebe and H.S. Saini, 1997. Early signs of disruption of wheat anther development associated with the induction of male sterility by meiotic-stage water deficit. Sex Plant Reprod., 10: 40-48.

Majd, A. and S. Jafari, 1996. Effects of salt stress on development of vegetative and generative organs and yield of Amygdalus two cultivars. M.Sc. Thesis, Tarbiat Modares University.

McRae, D.H., 1985. Advances in chemical hybridization. Plant Breed. Rev., 3: 169-191.

Morgan, J.M., 1980. Possible role of abscisic acid in reducing seed set in water-stressed wheat plants. Nature, 289: 655-657.

Moss, G.I. and L.A. Downey, 1971. Influence of drought stress on female gametophyte development in corn (*Zea mays*) and subsequent grain yield. Crop Sci., 11: 368-373.

Namuco, O.S. and J.C. O'Toole, 1986. Reproductive stage water-stress and strelity.1. effect of stress during meiosis. Crop Sci., 26: 317-321.

O'Toole, J.C. and T.B. Moya, 1981. Water deficit and yield on upland rice. Field Crop Res., 4: 247-259.

Sage, T.L. and B.D. Webster, 1990. Seed abortion in *Phaseolus vulgaris* L. Bot. Gaz., 151: 167-175.

Saini, H.S. and D. Aspinall, 1981. Effect of water deficit on sporogenesis in wheat (*Triticum aestivum*). Ann. Bot., 48: 623-633.

Saini, H.S., M. Sedgley and D. Aspinall, 1984. Developmental anatomy in wheat of male sterility induced by heat stress, water deficit or abscisic acid. Aust. J. Plant Physiol., 11: 243-253.

Sato, K., 1954. Relationship between rice crop and water. Jpn. J. Breed, 4: 264-289.

Sheoran, I.S. and H.S. Saini, 1996. Drought-induced male sterility in rice: changes in carbohydrate levels and enzyme activities associated with the inhibition of starch accumulation in pollen. Sex Plant Reprod., 9: 161-169.

Skazkin, F.D. and I.G. Zavadskaya, 1957. On the influence of soil moisture deficiency and nitrogen nutrition on microsporogenesis in barely. Dokl. Akad. Nauk. SSSR., 117: 240-242.

Sun, K., K. Hunt and B.A. Hauser, 2004. Ovule abortion in Arabidopsis triggered by stress. Plant Physiol., 135: 2358-2367.

Wells, S.A. and S. Dubetz, 1966. Reaction of barley varieties to soil water stress. Can. J. Plant Sci., 46: 507-512.

Westgate, M.E. and J.S. Boyer, 1986. Reproduction at low silk and pollen water potentials in maize. Crop Sci., 26: 951-956.

Westgate, M.E. and C.M. Peterson, 2004. Flower and pod development in water-deficient soybeans (*Glycine max* L. Merr.). J. Exp. Bot., 44: 109-117.

Young, L.W., R.W. Wilen and P.C. Bonham-Smit, 2004. High temperature stress of *Brassica napus* during flowering reduces micro and megagametophyte fertility, induces fruit abortion and disrupts seed production. J. Exp. Bot., 55: 458-495.

Zhang, H.X. and E. Blumwald, 2001. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nat. Biotechnol., 19: 765-768.

Zhu, J.K., 2002. Plant salt tolerance. Trends Plant Sci., 6: 66-71.