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A normal Micro- and Megagametophyte Development in *Brassica napus* Induced by High Salinity

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Abstract: A major factor impairing worldwide agricultural productivity is salinity, which is believed to affect nearly one-fifth of the world's irrigated land and causes 10^7 irrigated hectares to be abandoned each year. In canola, the effect of salt stress on reproduction was examined using plants grown in greenhouse. Structural and functional abnormalities in reproductive organs were observed when plants treated with NaCl solution (EC = 12 dS m^{-1}). Floral primordia, number of flowers, siliques, seeds per siliques and weight of 1000-seed were reduced in stressed plants. SEM studies showed that anthers filled with pollen corpses. During ovule abortion, gametophyte cells became more vacuolated. The resources conserved by reducing plant fertility, thereby, can be shunted into processes that permit plants to acclimate to stressful environmental conditions.

Key words: Salt stress, gametophyte fertility, canola, SEM studies, ovule development

INTRODUCTION

Productivity in agricultural ecosystem is severely reduced by various biotic and abiotic stresses (Boyer, 1982). The extent to which crop productivity is affected depends on the stage of development at which the plants encounter stress (Salter and Goode, 1967). Among these stages, reproductive development from meiosis in the spore mother cells to fertilization and early seed establishment is extremely sensitive to various stresses, such as drought (Salter and Goode, 1967; O'Toole and Moya, 1981; Saini and Aspinall, 1981), heat (Satake and Yoshida, 1978; Saini and Aspinall, 1982), cold (Hayase *et al*., 1969; Brooking, 1976; Lardon and Triboi-Blondel, 1994), flooding (Reddy and Mittra, 1985) and nutrient deficiencies (Sharma *et al*., 1987; Azouaou and Souvre, 1993). These stresses cause various structural and functional abnormalities in reproductive organs, leading to failure of fertilization or premature abortion of seed or fruit. Thus, the damage to productivity from the stress at this stage is particularly severe for crops in which the economic yield is the product of sexual reproduction as in cereals. Salinity stress probably ranks as the most important environmental factor limiting global crop productivity. In many crops, reproductive development is the most salinity-stress-sensitive period after seed germination and seedling establishment has been accomplished (Salter and Goode, 1967). 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. Salinity stress interferes with reproductive success of plants by arresting the development of the male

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gametophyte and sometimes, the female gametophyte, preventing fertilization and/or inducing premature abortion of the fertilized ovule (Sheoran and Saini, 1996). The objectives of this research were to determine the anatomical details of micro and megagametophyte and also seed and fruit production in stressed canola (*Brassica napus* L.).

MATERIALS AND METHODS

The experiment was conducted in Faculty of Science, Islamic Azad University, Mashad Branch, Iran (2006). *Brassica napus*, cultivar Okapi, was used for this study. The seeds were supplied by the Agricultural Research Center of Khorasan, Iran. Experiments on sowing seeds in five treated groups were conducted in greenhouse. There were a control group and four other groups which were treated with salt solutions of 3, 6, 9 and 12 dS m^{-1}. Plant tissues analyzed in this experiment were pistils at early stage, when megagametogenesis was complete but fertilization had not yet occurred and pollen from control-grown or treated plants was collected from flowers of approximately the same age. Siliques containing seeds were collected and silique number, seed number, weight of 1000 seed, weight of 1000 silique were also determined from ten randomly selected plants. For paraffin sections, flowers were fixed overnight in 10 formalin, 5 acetic acid and 50% ethanol. Samples were dehydrated in a graded ethanol series then embedded in paraplast. Samples were sectioned at 7 m intervals, then stained with hematoxylin and eosine. An light microscope (Zeiss) equipped with a Kodak camera captured microscopic images. For SEM studies, pollen grains were mounted on aluminium stubs, sputter-coated with gold in a polaron E 5100 sputter coater and viewed in a LEO SEM.

Data Analysis

To test the significance of number of flowers, siliques, seeds, data were statistically analyzed using analysis of variance (ANOVA).

RESULTS

In canola plants, the addition of NaCl to the pots, induced numerous symptoms, including reduced growth and decreased fertility. On a macroscopic scale, decreased fecundity was noticeable when fruit length declined (Fig. 1), since canola seed produces signals that regulated expansion. Fruits

Fig. 1: Stress reduces canola fruit size. (a) The fruits of a healthy canola plant were plump and uniform in size. (b) Treated plant with NaCl solution (EC =12 dS m^{-1})

containing predominantly aborted ovules and embryos were narrower and shorter than healthy ones. The number of floral primordia, flowers, seeds per fruit, siliques, length of siliques and weight of 1000-seed were reduced with increasing of salinity levels (Fig. 1-6). The addition of different

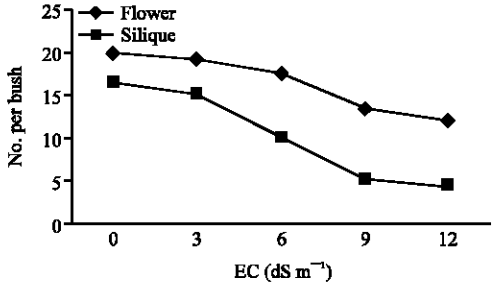

Fig. 2: Salt stress reduced number of flower and silique per bush in canola

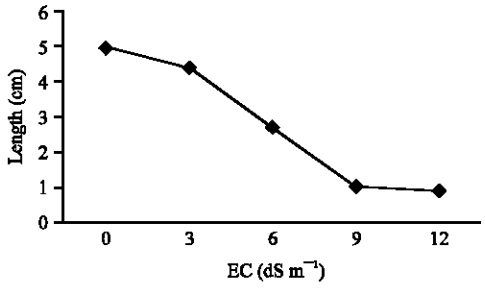

Fig. 3: Salt stress reduced length of siliques in canola

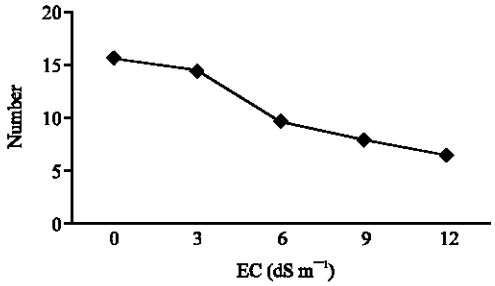

Fig. 4: Salt stress reduced number of seeds in per silique in canola

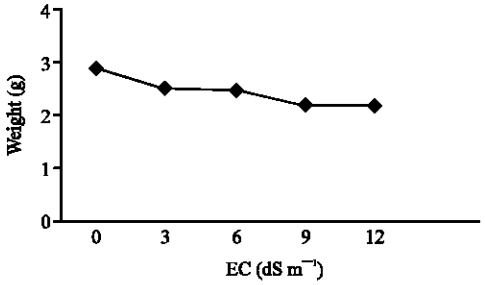

Fig. 5: Salt stress reduced weight of 1000-seed in canola

concentrations of NaCl to the soil increased both the osmoticum and the concentration of Na^+ and Cl^- ions. The reduction in plant fertility might be caused either by increases in ion concentration or by decreases in water potential. It seems unlikely that Na^+ ions would be transported to ovules and reach toxic levels. SEM studies showed that, the pollen grains of canola are oval and exine with reticulated ornamentation and triporate (Fig. 7a, b). In treated plants, pollen grains were wrinkled and abnormal (Fig. 7b). Developing microspores are very sensitive to salt stress (Namuco and O'Toole, 1986). Healthy canola microsporocytes each contained a single nucleus that was densely stained by the histochemical dyes (Fig. 8a, b), indicating rapid growth and development. The cells that were salt stressed when they were undergoing microsporogenesis did not mature into viable pollen grains. Instead, these cells became consicuounsly vacuolated and the majority of them senesced within 2d,

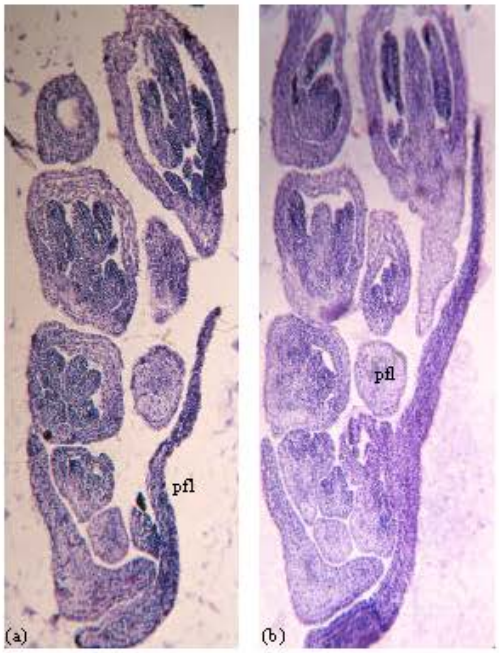

Fig. 6: Salt stress reduced the number of floral primordial (pfl.) in stressed plants (b), in comparison with control plants (a)

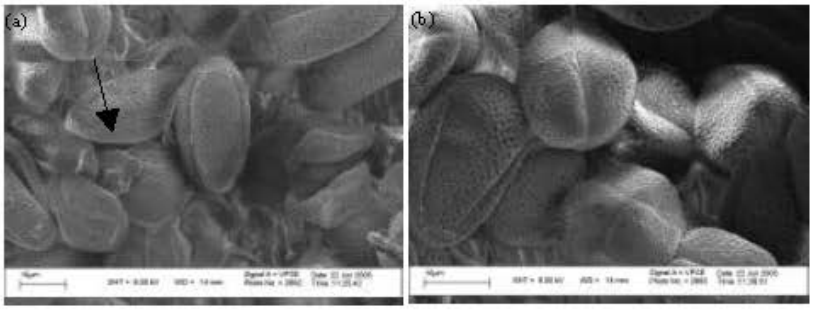

Fig. 7: Scanning electron microscopy images of pollen grains with reticulated ornamentation and triporate. (a) Pollen grains of healthy plants and (b) Wrinkled pollens in stressed plants (arrow)

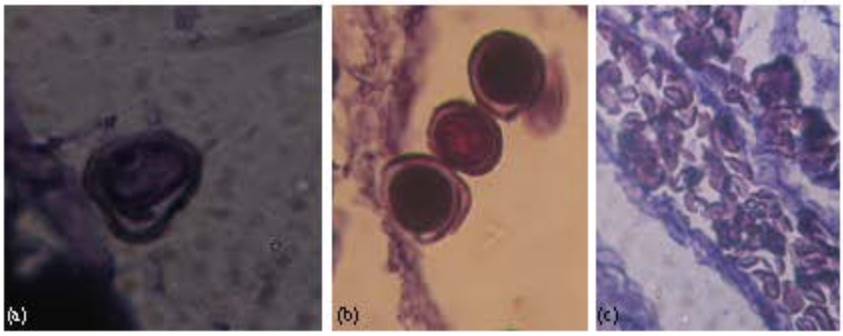

Fig. 8: Healthy pollen grains had identified nucleus (a) and cytoplasm was dispersed throughout the cell (b). Salt stress disrupted the normal development of canola pollen grains (c)

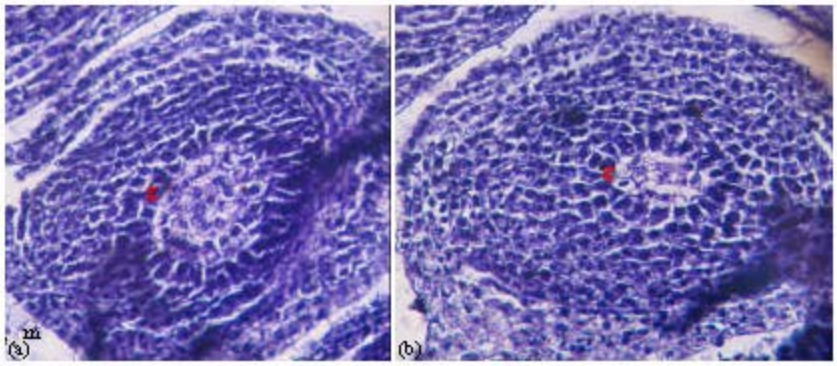

Fig. 9: Salt stress disrupted the normal development of canola ovules. When compared to healthy ovules (a), in stressed plants, during ovule abortion, gametophyte (g) cells became more vacuolated (b)

leaving anthers filled with pollen corpses (Fig. 8c). The growth of filaments causes the stamens to brush against the stigma and coat it with pollen. In healthy plants, stamen filaments and petals 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 slowly than their counterparts, they did manage to pollinate the stigma. This slower growth delayed pollination, although this delay did not prevent fertilization of female gametophytes. In healthy ovules, the megaspore mother cell undergoes meiosis yielding a large megaspore. This cells undergoes further cell divisions and differentiation to form a mature megaspore. When plants were stressed during or just to meiosis, meiosis concluded and megaspores were produced, but further development of these into female gametophytes was blocked. Once gametophyte development ceased, gametophytic nuclei dissipated and cells collapsed. During ovule abortion, gametophyte cells became more vacuolated(Fig. 9b). While environmental stress caused most female gametophytes to abort during development, a fraction of them remained viable. In plants stressed just prior to anthesis, pistils were pollinated and fertilization occurred in one-quarter of the ovules. The majority of the resulting zygotes formed four-cell or globular-stage embryos but did not develop further. Stressed embryos commonly were more vacuolated than healthy counterparts. The condensed cytoplasm found in some endothelial cells revealed that those cells underwent Programmed Cell Death (PCD). Since, these cells normally transport photosynthate into the gametophyte, their demise should reduce transport rates.

DISCUSSION

Because of the substantial plant resources required for reproduction, plants regulate pollen, ovule and seed development in response to changing environmental conditions. Under harsh environmental conditions, developing pollen, ovules and/or embryos abort in many seed-bearing plants. The research described here details how a plant, canola, curtails plant reproduction in response to stress. While stress delayed filament growth and pollination, ovule abortion did not result simply from a lack of synchronization between pollination and ovule development. Ovules remained viable for an additional 2 to 3 days in emasculated plants (data not shown). Thus, ovule abortion was complete or nearly complete at a time when gametophytes in healthy plants remained fertile and receptive. The duration of salt stress affected both ovule and pollen development in canola, resulting in significant decreases in seed production. Depending on the stage of development, stress had three effects on female reproduction: (1) gametophyte differentiation was derailed, (2) mature gametophytes underwent PCD, or (3) fertilized ovules senesced. In a number of crop plants, including bean, rapeseed, maize and soybean, stress induces mature gametophytes to undergo PCD (Moss and Downey, 1971; Sage and Webster, 1990; Kokubun *et al*., 2001; Young *et al*., 2004). Thus, adverse environmental conditions can cause plants to suspend normal development of gametophytes, embryos and pollen grains but the phenotype of abortion depends upon the developmental stage at which stress was experienced. In many plant species, salt stress attenuate seed st or yields (Boyer, 1982). Under most growing conditions, sufficient numbers of ovules are initiated to maximize reproduction. Since most plants do not experience optimal growth conditions, inevitably some of these ovules abort. At times of environmental stress, the nutritional demand of reproduction frequently exceeds the carbon and nitrogen resources allocated to the ovule. Consequently, the number of ovules initiated often exceeds the capacity of the gynoecium or, by extension, the plant to provide adequate nutrition for them all (Lee and Bazzaz, 1986). Following water stress, the rate of photosynthesis dramatically drops in maize (Westgate and Boyer, 1985a), thus reducing sugar transport into the embryo sac, which limits resources for seed development (Schussler and Westgate, 1995). In healthy plants, manual removal of some developing ovules decreases the overall rate of abortion by providing more photosynthate for surviving ovules. For example, the total number of cucurbit ovules that matured into seeds remained steady but the rate of ovule abortion declined when a portion of the ovules were surgically removed (El-Keblawy and Lovell-Doust, 1999). The maize and cucurbit examples are two of many instances where the demand for photosynthate limits seed production. Resource limitation, however, is only one variable affecting reproductive success. This point is elegantly illustrated in experiments on maize plants that were artificially supplemented with sucrose during seed development. Westgate and Boyer (1985b) utilized maize to study the effects of water stress on plant reproduction. In these experiments, reduction in watering gradually imposed osmotic stress over a period of days, which allowed plants to acclimate to this stress. In present experiments, osmotic conditions were changed rapidly so, plants did not have the opportunity to acclimate to osmotic stress. Nonetheless, canola exhibited many of the same symptoms observed in other plant systems. In maize, transport of photosynthate into seedlings was partially corrected by stem-infusion of sucrose (Zinselmeier *et al*., 1999; Fisher and Cash-Clark, 2000). If sucrose deficiency were the sole obstacle to maize reproduction, then stem infusion of sucrose should completely restore fertility. Insufficient transport of carbohydrates into the ovary, as opposed to sugar deficiency, forces seeds to abort (Schussler and Westgate, 1995). Defects in seed development and sugar transport derive at least in part from reduced acid invertase activity and carbohydrate transport into ovules. In these plants, sucrose accumulated in the apoplast between the maternal tissue and the developing embryo. Thus, carbohydrate transport into the embryo sac, as opposed to sucrose availability, limited seed formation. Defects in carbohydrate transport into the embryo sac are not limited to maize. In other species, photosynthate transport becomes occluded in

aborting ovules concurrent with a massive proliferation of integument tissue (Rappaport *et al*., 1950; Savenchko, 1960; Lacey *et al*., 1997). In plant where the maternal tissue proliferation, the ovule remains an excellent sink tissue, even though the transfer of assimilates into developing gametophytes or embryos has ceased. In beans, as in a number of other plants, hormones were implicated in the regulation of sink strength and seed abortion (Tamas *et al*., 1979, 1986). These results indicate that hormones produced by maternal tissues regulate the transfer of photosynthate into gametophytes and embryos. In stressed canola plants, very few seeds per siliques matured, even though one-quarter of the ovules were fertilized and produced zygotes. These zygotes formed globular embryos, but the majority of them senesced at this stage of embryogenesis. Similar to what was observed in maize, failure in canola embryogenesis may result from a cessation of nutrient transfer into the embryo sac. To determine if this hypothesis is tenable, the movement of assimilates into canola embryo sacs needs to be measured in healthy and stressed plants. While male meiosis is highly sensitive to stress (Saini, 1997), this does not appear to be true with female meiosis. When canola ovules were stressed during meiosis, they completed this process and formed megaspores. However, megagametogenesis arrested before the mitotic divisions were completed. Thus, megagametogenesis was inhibited by environmental stress. These results are consistent with that hypothesis that one or more stress-induced regulators curtailed reproductive development.

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REFERENCES

Azouaou, Z. and A. Souvré, 1993. Effects of copper deficiency on pollen fertility and nucleic acids in the durum wheat anther. Sex Plant Reprod., 6: 199-204.

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

Brooking, I.R., 1976. Male sterility in *Sorghom bicolor* L. induced by low night temperature. I. Timing of the stage of sensitivity. Aust. J. Plant Physiol., 3: 589-596.

El-Keblawy, A. and J. Lovell-Doust, 1999. Maternal effects in the progeny generation in zucchini, *Cucurbita pepo* (Cucurbitaceae). Int. J. Plant Sci., 160: 331-339.

Fisher, D.B. and C.E. Cash-Clark, 2000. Gradients in water potential and turgor pressure along the translocation pathway during grain filling in normally watered and water-stressed wheat plants. Plant Physiol., 123: 139-147.

Hayase, H., T. Satake, I. Nashiyama and N. Ito, 1969. Male sterility caused by cooling treatment at the meiotic stage in rice plants. II. The most sensitive stage to cooling and fertilizing ability of pistils. Prod. Crop Sci. Soc. Jpn., 38: 706-711.

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.

Lacey, E.P., S. Smith and A.L. Case, 1997. Parental effects on seed mass: Seed coat but not embryo/endosperm effects. Am. J. Bot., 84: 1617-1620.

Lardon, A. and A.M. Triboi-Blondel, 1994. Freezing injury to ovules, pollen and seeds in winter rape. J. Exp. Bot., 45: 1177-1181.

Lee, T.D. and F.A. Bazzaz, 1986. Maternal regulation of fecundity: Nonrandom ovule abortion of *Cassia fasciculate* Michx. Oecologia, 68: 459-465.

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 sterility. 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.

Rappaport, J., S. Satina and A.F. Blakeslee, 1950. Ovular tumors associated with incompatible crosses in *Datura*. Am. J. Bot., 37: 576-586.

Reddy, M.D. and B.N. Mittra, 1985. Effect of complete plant submergence at different growth stages on grain yield, yield components and nutrient content of rice. Plant Soil, 86: 379-386.

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

Saini, H.S., 1997. Effects of water stress on male gametophyte development in plants. Sex Plant Reprod., 10: 67-73.

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. and D. Aspinall, 1982. Abnormal sporogenesis in wheat (*Triticum aestivum* L.) induced by short periods of high temperature. Ann. Bot., 49: 835-846.

Salter, P.J. and J.E. Goode, 1967. Crop Responses to Water at Different Stages of Growth (Research review number 2) Common Wealth Agricultural Bureaux. Farnham Royal, UK.

Satake, T. and S. Yoshida, 1978. High temperature induced sterility in indica rices at flowering. Jap. J. Crop Sci., 47: 6-17.

Savenchko, M.I., 1960. Anormalies in the structure of angiosperm ovules. Bot. Sci., 130: 15-17.

Schussler, J.R. and M.E. Westgate, 1995. Assimilate flux determines kernel set at low water potential in maize. Crop Sci., 35: 1074-1080.

Sharma, P.N., C. Chatterjee, C.P. Sharma and S.C. Agarwala, 1987. Zinc deficiency and anther development in maize. Plant Cell Physiol., 28: 11-18.

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.

Tamas, I.A., D.H. Wallace, P.M. Ludford and J.L. Ozbun, 1979. Effect of older fruits on abortion and abscisic acid concentration of younger fruits in *Phaseolus vulgaris* L. Plant Physiol., 64: 620-622.

Tamas, I.A., J.L. Kock, B.K. Mazur and P.M. Davies, 1986. Auxin effects on the correlative interaction among fruits in *Phaseolus vulgaris L.* Proceeding of the Plant Growth Regul. Society Am., pp: 208-215.

Westgate, M.E. and J.S. Boyer, 1985a. Carbohydrate reserves and reproductive development at low leaf water potential in maize. Crop Sci., 25: 762-769.

Westgate, M.E. and J.S. Boyer, 1985b. Osmotic adjustment and inhibition of leaf, root, stem and silk growth at low water potentials in maize. Planta, 104: 540-549.

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.

Zinselmeier, C., B.R. Jeong and J.S. Boyer, 1999. Starch and the control of kernel number in maize at low water potentials. Plant Physiol., 121: 25-35.