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Incorporation of Polyamines in the Priming Media Enhances the Germination and Early Seedling Growth in Hybrid Sunflower (*Helianthus annuus* L.)

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ABSTRACT

A laboratory study was conducted to evaluate the benefits (if any) associated with the incorporation of polyamines in the priming media for hybrid sunflower achene priming. Achenes were subjected to hydropriming for 24 h and in the solutions containing 10 mg L^{-1} spermidine and putrescine for 24 h. All the achene priming treatments resulted in improved germination and early seedling growth. Priming in spermidine solution resulted in lower time to start emergence, time taken 50% emergence and mean emergence time and energy of emergence, emergence index, root and shoot length and seedling fresh and dry weight than all other treatments including control. However, hydroprimed achenes resulted in maximum final emergence and leaf score; while priming in putrescine solution resulted in maximum number of roots.

Key Words: Priming; Sunflower; Polyamines; Germination; Achenes

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil seed crops in Pakistan. It is a short duration crop (90-110 days), can be grown twice in a year. It is fully fit in our cropping system and can be grown without causing displacement of any major crop. One of the major obstacles to high yield and production is the lack of synchronized crop establishment in sunflower (Mwale *et al.,* 2003). The seeds are occasionally sown in seedbeds having unfavorable moisture, because of the lack of rainfall at sowing time, results in poor and unsynchronized seedling emergence (Mwale *et al.,* 2003).

In recent years, a lot of work has been done on the invigoration of seeds to improve the germination rate and uniformity of growth and reduce the emergence time of many vegetables and some field crops (Basra *et al.,* 2003). Furthermore, the invigoration persists under less than optimum conditions such as salinity (Muhyaddin & Weibe, 1989), excessively high and low temperature (Pill & Finch-savage, 1988; Bradford *et al.,* 1990). Seed invigoration treatments such as hydropriming, osmopriming, hardening, matripriming and growth regulators have been successfully employed in many parts of the world.

Seed priming has been successfully demonstrated to improve germination and emergence in many crops, particularly seeds of vegetables and small seeded grasses (Heydecker & Coolbaer, 1977; Bradford, 1986). The beneficial effects of priming have also been demonstrated for many field crops such as wheat, sugar beet, maize, soybean and sunflower (Parera & Cantliffe, 1994; Singh, 1995; Khajeh-Hosseini *et al.,* 2003; Sadeghian & Yavari, 2004). Dharmalingam and Basu (1990) reported beneficial effect of a hydration-dehydration seed treatment on germination of sunflower. Rao *et al.* (1987) reported that primed Brassica seeds may reduce the risk of poor stand establishment in cold and moist soils. However, Singh and Rao (1993) stated that KNO_3 effectively improved germination, seedling growth and seedling vigor index of the seeds of sunflower varieties with low germination.

Polyamines are known to have profound effects on plant growth and development (Watson & Malmberg, 1998). Polyamines, being cationic in nature, can associate with anionic components of the membrane such as phospholipids thereby stabilizing the bilayer surface and retarding membrane deterioration under stress conditions (Basra *et al.,* 1994). There is now conclusive evidence that polyamines (PAs) accumulate in plants and are involved in their protection against various environmental stresses (Bouchereau *et al.,* 1999; He *et al.,* 2002). Even if polyamines accumulate, this does not mean that they are involved in stress protection, especially as the role of polyamines may depend on their cellular localization and whether they are free, bound to proteins or conjugated to phenolic acids (Bouchereau *et al.,* 1999). However, there are many reports that indicate that stress tolerance of plants is correlated with their capacity to enhance the synthesis of PAs under stress (Bouchereau *et al.,* 1999; Kasukabe *et al.,* 2004). Of the different polyamines, spermidine (Spd) is more closely associated with stress tolerance of plants than are putrescine (Put) and spermine (Spm) (Bouchereau *et al.,* 1999; He *et al.,* 2002; Martı´nez-Téllez *et al.,* 2002).

Working with some wheat cultivars, El-Shintinawy (2000) found that salt stress enhanced the accumulation of Spd and Spm concentration associated with a decrease in Put in the wheat cultivars. In contrast, in other wheat cultivars, it was found that salt stress increased the concentration of Put and decreased that of Spm (Simon-Sarkadi *et al.,* 2002). However, in rice Lin and Kao (1995) found that increasing NaCl concentration caused a decrease in free Put concentration, but an increase in Spd concentration in a salt intolerant rice cultivar, cv. Taichung Native 1. Furthermore, in pea (Anderson & Martin, 1973) and barley (Smith, 1973), neither Put nor Spd accumulated in plants when exposed to salinity stress. These reports indicate that the individual polyamines have different roles during the response of plants to salt stress.

Incorporation of plant growth regulators during pre-soaking, priming and other pre-sowing treatments have improved seed performance in many crops (Jeong *et al*., 1994). But still no study has been conducted to explore the potential of polyamines incorporation in the priming media. The present study was, therefore carried out with the objective to investigate the benefits (if any) associated with the incorporation of polyamines in the priming medium for hybrid sunflower.

MATERIALS AND METHODS

Achene materials. Sunflower hybrid Hysun-33 was used in the present study. The Achenes were obtained from Oilseed Research Institute, Ayyub Agricultural Research Institute, Faisalabad Pakistan. The initial achene moisture contents were 8.13% (on dry weight basis).

Achene priming. For priming with polyamines, the achenes were soaked in aerated solutions of 10 g L^{-1} spermidine and putrescine for 24 h. The ratio of achene weight to solution volume was 1:5 (g/mL) (Basra *et al.,* 2004). For hydropriming, the sunflower achenes (250 g) were soaked in aerated distilled water for 24 h (Hussain *et al.,* 2006). The ratio of achene weight to solution volume was 1:5 (g mL^{-1}) (Basra *et al.,* 2004).

Post treatment operations. After treatment, achenes were given three surface washings with distilled water and re-dried to original weight with forced air under shade at 27 ± 3°C (Basra *et al.,* 2002). These achenes were then sealed in polythene bags and stored in refrigerator at 5°C before further use.

Vigor evaluation. Control and treated achenes were sown in 5 kg plastic pots containing moist acid/water washed sand and placed in a net-house. The number of emerged achenes was recorded daily according to the seedling evaluation Handbook of Association of Official Seed Analysts (1990) until a constant count was achieved. Time taken to 50% emergence of seedlings (E_{50}) was calculated according to the following formulae of Coolbear *et al.* (1984) modified by Farooq *et al.* (2005):

$$E_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

Where, N is the final number of emerged achenes and n_i and n_j the cumulative number of achenes emerged by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981) as under:

$$MET = \frac{\sum Dn}{\sum n}$$

Where, n is the number of achenes, which were emerged on day D and D is the number of days counted from the beginning of emergence.

Emergence index (EI) was calculated as described in the Association of Official Achene Analysts (1983) as the following formulae:

$$EI = \frac{\text{No. of emerged seeds}}{\text{Days of first cout}} + --- + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}$$

Energy of emergence (EE) was recorded on the fourth day after plantation. The percentage of emerging seeds 4 d after plantation is relative to the total number of seeds tested (Ruan *et al.,* 2002). On the fifteen day after emergence, the seedlings were tested for vigor after carefully removing from the sand. Number of roots and leaves, shoot and root length of 5 randomly selected seedlings were recorded per replicate and averaged. Seedling fresh weight was determined immediately after harvest, whereas dry weight was taken after drying at 70°C for 7 d.

RESULTS

Achene priming techniques significantly reduced the time to start of germination, E_{50} and MET compared with control (Fig. 1a, b & 2a), however incorporation of spermidine in the priming medium resulted in the earliest germination start and lower values of E_{50} and MET. Although all the priming treatments resulted in improved emergence percentage, emergence energy and emergence index compared with untreated Achenes (Fig. 2b & 3a, b), maximum emergence percentage was observed in hydroprimed Achenes but priming in spermidine solution resulted in maximum emergence energy and emergence index than all other treatments (Fig. 3a, b).

All the achene priming techniques resulted in improved root and shoot length, seedling fresh and dry weight and leaf score compared with untreated achenes (Fig. 4a, b; 5a, b & 6a). Maximum root and shoot length and seedling fresh and dry weight were recorded in priming in spermidine solution that was similar to that of hydropriming

Fig. I. Influence of Seed priming treatments on the (a) time to start emergence and (b) time to 50% emergence ± s.e

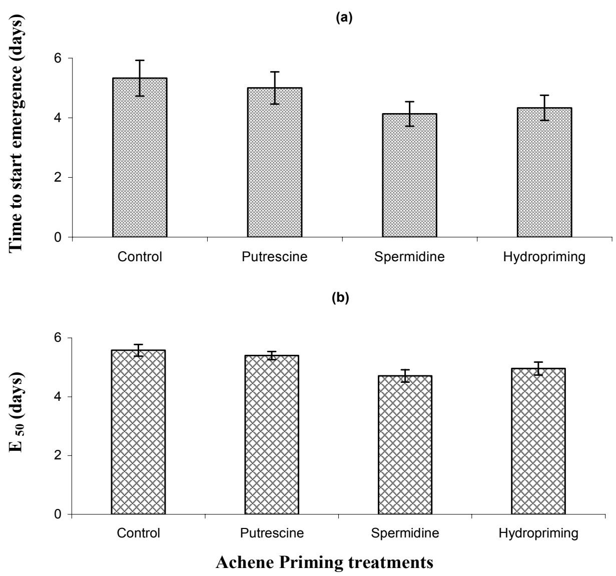


Fig. 3. Influence of Seed priming treatments on the on the (a) emergence energy (EE) and (b) emergence index (EI) ± s.e

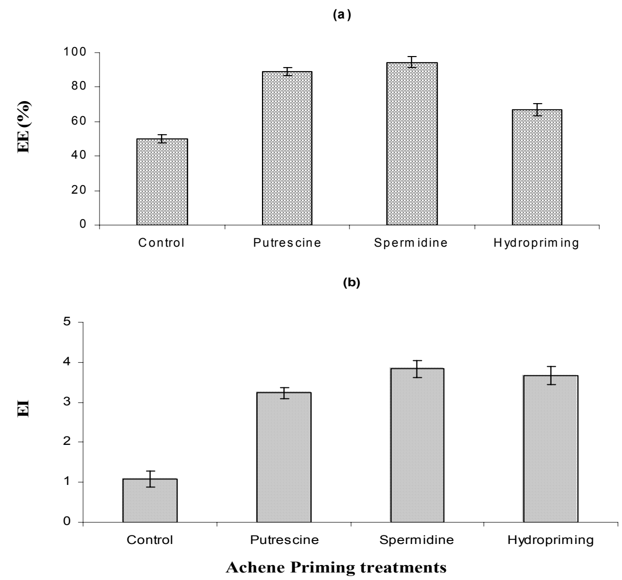


Fig. 2. Influence of Seed priming treatments on the on the (a) mean emergence time and (b) final emergence percentage ± s.e

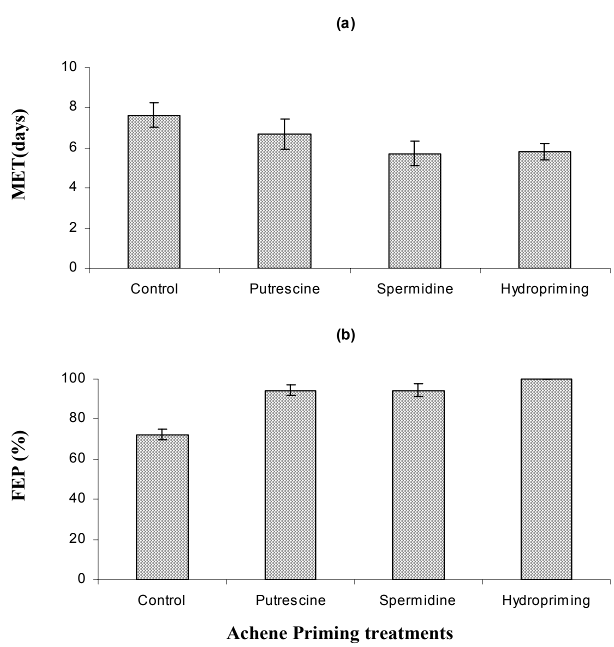
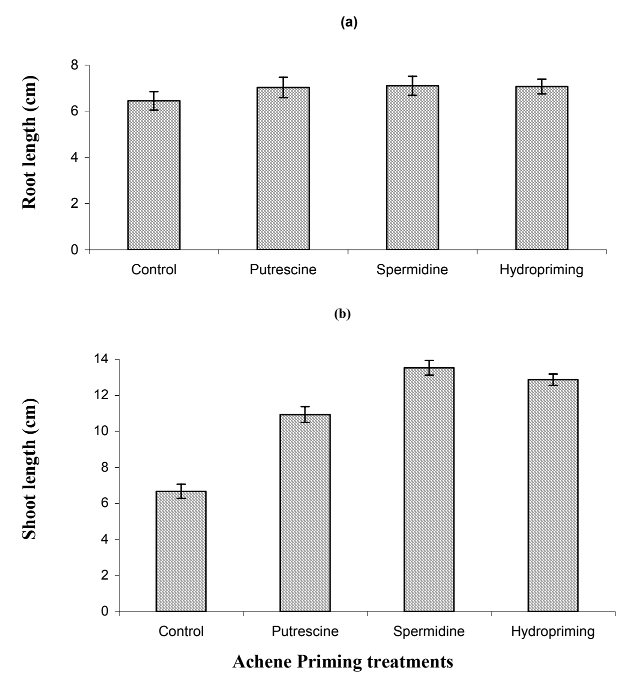


Fig. 4. Influence of Seed priming treatments on the on the (a) Root length and (b) Shoot length ± s.e



and priming in putrescine solution in case of root length and with priming in putrescine solution in case of seedling fresh and dry weight. Maximum leaf score was recorded in hydroprimed achenes, which was similar to all other treatments except control (Fig 6a).

However, minimum number of roots were observed in hydroprimed achenes compared with all other treatments, while maximum roots were noted in achenes primed in putrescine solution (Fig 6b).

DISCUSSION

It is revealed from the present study that different priming techniques can enhance the germination and early seedling growth in hybrid sunflower. Primed achenes had higher vigor (Basra *et al*., 2002), which resulted in earlier

Fig. 5. Influence of Seed priming treatments on the on the (a) Seedling fresh weight and (b) Seedling dry weight± s.e

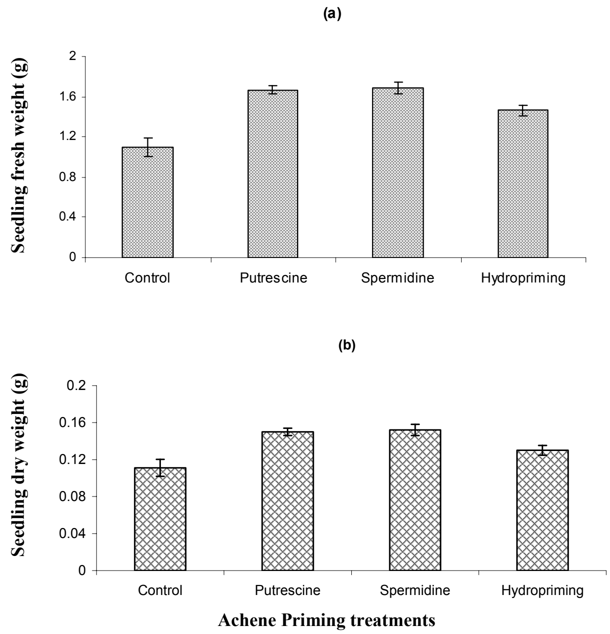
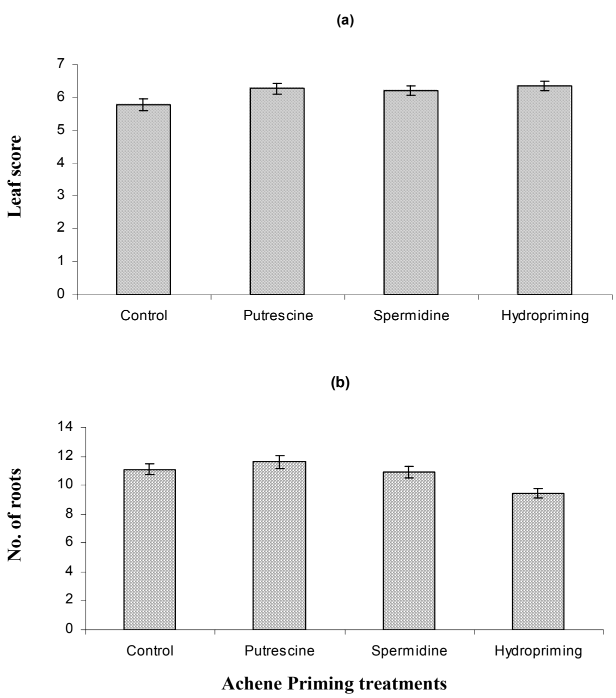


Fig. 6. Influence of Seed priming treatments on the on the (a) Leaf score and (b) No. of roots± s.e



start of emergence (Basra *et al.,* 2002). Positive correlation between Achene vigor and field performance had also been found in rice (Yamauchi & Winn, 1996). Achene priming techniques not only resulted in earlier and more uniform emergence (as is clear from lower vales of time to start emergence, E_{50} & MET) but the mergence percentage, energy of emergence and emergence index were also improved. Achene priming in the spermidine solution was the most effective compared with other priming techniques. The earliness in emergence might be the result of increased rate of metabolisms. Higher mergence percentage, energy of emergence and emergence index might be attributed to embryo development and/or leaching of emergence inhibitors (Yamauchi & Winn, 1996). Basra *et al.* (1994) reported that priming of onion seeds increased the polyamines in aged onion seeds. They correlated the increased polyamines with the improved vigor. This gives an idea that polyamine seed treatments contributed towards enhanced build up of polyamines that resulted in improved vigor and early seedling growth. Spermidine (Spd) incorporation in the priming medium performed the best, which is in support with the earlier findings of (Bouchereau *et al.,* 1999; He *et al.,* 2002; Martı´nez-Téllez *et al.,* 2002). Gray *et al.* (1984) concluded that osmopriming of a slowly germinating stock improved the percentage seedling emergence compared with untreated seeds. Priming also reduced the mean emergence time but had no effect on the spread of emergence time of parsnip seeds. Nerson and Govers (1986) subjected the seeds of muskmelon to salt priming and found that 2 - 3% solutions of $KH_2PO_4 + KNO_3$ (1:1) for 1 - 5 days significantly increased the emergence rate, synchronization and percentage. Kathiresan *et al.* (1984) reported enhanced field emergence from ascorbic acid NaCl and $CaCl_2$ treated sunflower Achenes. In another experiment, hydropriming for 6 days at 15ºC, in *Helichrysum bracteatum* accelerated emergence (Grzesik & Nowak, 1998), while Pill and Necker (2001) found that hydropriming failed to improve emergence in common Kentucky bluegrass seeds. These findings support the earlier work of Basra *et al.* (2002) and Shahbaz *et al.* (1998), who reported enhanced seedling establishment in hydroprimed canola and sunflower, respectively.

From the present study, it is be concluded that the effectiveness of seed priming can be enhanced by the incorporation of polyamines in the priming medium, spermidine being the most effective.

REFERENCES

Anderson, J.N. and R.O. Martin, 1973. Identification of cadaverine in *Pisum sativum*. *Photochem.*, 12: 443–6

Association of Official Seed Analysis (AOSA), 1983. *Seed Vigor Testing Handbook*. Contribution No. 32 to the handbook on Seed Testing. Association of Official Seed Analysis, Springfield, IL

Association of Official Seed Analysis (AOSA), 1990. Rules for testing seeds. *J. Seed Technol.,* 12: 1–112

Basra, A.S., B. Singh and C.P. Malik, 1994. Priming-induced changes in polyamine levels in relation to vigor of aged onion seeds. *Bot. Bull. Academia Sin.*, 35: 19–23

Basra, S.M.A., M.N. Zia, T. Mehmood, I. Afzal and A. Khaliq, 2002. Comparison of different invigoration techniques in wheat (*Triticum aestivum* L.) seeds. *Pakistan J. Arid Agric.,* 5: 11–6

Basra, S.M.A., M. Farooq and A. Khaliq, 2003. Comparative study of pre-sowing seed enhancement treatments in indica rice (*Oryza sativa* L.). *Pakistan J. Life Soc. Sci.,* 1: 5–9

Basra, S.M.A., M. Farooq, K. Hafeez and N. Ahmad, 2004. Osmohardening: A new technique for rice seed invigoration. *Int. Rice Res. Notes,* 29: 80–1

Bouchereau, A., A. Aziz, F. Larher and M. Tanguy, 1999. Polyamines and environmental challenges: Recent development. *Pl. Sci.*, 140: 103–25

Bradford, K.J., 1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *Hort. Sci.*, 21: 1105–12

Bradford, K.J., J.J. Steiner and S.E. Trawatha, 1990. Seed priming influence on germination and emergence of pepper seed lots. *Crop Sci.,* 30: 718–21

Coolbear, P., A. Francis and D. Grierson, 1984. The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds. *J. Exp. Bot.*, 35: 1609–17

Dharmalingam, C. and R.N. Basu, 1990. Maintance of viability and vigour in sunflower. *Seed Sci. Res.,* 18: 15–24

Ellis, R.A. and E.H. Roberts, 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci. Technol.*, 9: 373–409

El-Shintinawy, F., 2000. Photosynthesis in two wheat cultivars differing in salt susceptibility. *Photosynthetica*, 38: 615–20

Farooq, M., S.M.A. Basra, K. Hafeez and N. Ahmad, 2005. Thermal hardening: a new seed vigor enhancement tool in rice. *J. Integr. Pl. Biol.,* 47: 187–93

Gray, D., P.A. Brocklehurst, J.A. Steckel and J. Dearman, 1984. Priming and pregermination of parsnip (*Pastinaca sativa* L.) seed. *Hort. Sci.,* 59: 101–8

Grzesik, M. and J. Nowak, 1998. Effects of matriconditioning and hydropriming on (*Helichrysum bracteatum* L.) seed germination, seedling emergence and stress tolerance. *Seed Sci. Technol.*, 26: 363–76

He, L., K. Nada and S. Tachibana, 2002. Effects of spermidine pretreatment through the roots on growth and photosynthesis of chilled cucumber plants (*Cucumis sativus* L.). *J. Japanese Soc. Hort. Sci.*, 71: 490–8

Heydecker, W. and P. Coolbear, 1977. Seed treatments for improved performance - survey and attempted prognosis. *Seed Sci. Technol.*, 5: 353–425

Hussain, M., M. Farooq, S.M.A. Basra and N. Ahmad, 2006. Influence of seed priming techniques on the seedling establishment, yield and quality of hybrid sunflower. *Int. J. Agric. Biol.*, 8: 14–8

Jeong, Y.O., J.L. Cho and S.M. Kang, 1994. Priming effect of pepper (*Casicum annum* L.) as affected by aging and growth regulators treatments. *J. Korean Soc. Hort. Sci.,* 35: 407–14

Kasukabe, Y., L. He, K. Nada, S. Misawa, I. Ihara and S. Tachibana, 2004. Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic Arabidopsis thaliana. *Pl. Cell Physiol.,* 45: 712–22

Kathiresan, K., V. Kalyani and J.L. Ganarethinam, 1984. Effect of seed treatments on field emergence, early growth and some physiological processes of sunflower (*Helianthus annuus* L.). *Field Crop Res.*, 9: 215–7

Khajeh-Hosseini, M., A.A. Powell and I.J. Bingham, 2003. The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Sci. Technol.*, 31: 715–25

Lin, C.C. and C.H. Kao, 1995. Levels of endogenous polyamines and NaCl inhibited growth of rice seedlings. *Pl. Growth Regul.*, 17: 15–20

Martı´nez-Te´llez, M.A., M.G. Ramos-Clamont, A.A. Gardea and I. Vargas-Arispuro, 2002. Effect of infiltrated polyamines on polygalacturonase activity and injury response in zucchini squash (*Cucurbita pepo* L.). *Biochem. Biophys. Res. Comm.,* 295: 98–101

Muhyaddin, T. and H.J. Weibe, 1989. Effect of seed treatments with poly ethylene glycol (PEG) on emergence of vegetable seeds. *Seed Sci. Technol.,* 17: 49–56

Mwale, S.S., C. Hamusimbi and K. Mwansa, 2003. Germination, emergence and growth of sunflower (*Helianthus annuus* L.) in response to osmotic seed priming. *Seed Sci. Technol.*, 31: 199–206

Nerson, H. and A. Govers, 1986. Salt priming of muskmelon seeds for low temperature germination. *Scientia Hort.,* 28: 85–91

Parera, C.A. and D.J. Cantliffe, 1994. Pre-sowing seed priming. *Hort Rev.*, 16: 109–41

Paul, S.R. and A.K. Choudhary, 1991. Effect of seed priming with Salts on growth and yield of wheat under rainfed conditions. *Annl. Agric. Res.,* 12: 415–8

Pill, W.G. and W.E. Finch-Savage, 1988. Effects of combining priming and plant growth regulator treatments on the synchronization of carrot seed germination. *Annl. Appl. Biol.,* 113: 383–9

Pill, W.G. and A.D. Necker, 2001. The effects of seed treatments on germination and establishment of Kentucky bluegrass (*Poa pratensis* L.). *Seed Sci. Technol.,* 29: 65–72

Rao, S.C., S.W. Aker and R.M. Ahring, 1987. Priming Brassica seed to improve emergence under different temperatures and soil moisture conditions. *Crop Sci.*, 27: 1050–3

Ruan, S., Q. Xue and K. Tylkowska, 2002. The influence of priming on germination of rice (*Oryza sativa* L.) seeds and seedling emergence and performance in flooded soils. *Seed Sci. Technol.,* 30: 61–7

Sadeghian, S.Y. and N. Yavari, 2004. Effect of water-deficit stress on germination and early seedling growth in sugar beet. *J. Agron. Crop Sci.,* 190: 138–44

Shahbaz, A., M. Anwar and H. Ullah, 1998. Wheat seed pre-soaking for improved germination. *J. Agron. Crop Sci.,* 181: 125–7

Simon-Sarkadi, L., G. Kocsy and Z. Sebestye´ n, 2002. Effect of salt stress on free amino acid and polyamine content in cereals. *Acta Biologia Szegediensis*, 46: 73–5

Singh, B.G., 1995. Effect of hydration-dehydration seed treatments on vigour and yield of sunflower. *Indian J. Pl. Physiol.*, 38: 66–8

Singh, B.G. and G. Rao, 1993. Effect of chemical soaking of sunflower (*Helianthus annuus* L.) seed on vigour index. *Indian J. Agric. Sci.*, 63: 232–3

Smith, T.A. 1973. Amine levels in mineral deficient *Hordeum vulgaris* leaves. *Phytochem.,* 12: 2093–100

Watson, M.B. and R.L. Malmberg, 1998. Arginine decarboxylase (polyamine synthesis) mutants of Arabidopsis thaliana exhibit altered root growth. *Pl. J.*, 13: 231–9

Yamauchi, M. and T. Winn, 1996. Rice seed vigor and seedling establishment in anaerobic soil. *Crop Sci.*, 36: 680–6

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