

PRE-SOWING SALICYLATE SEED TREATMENTS IMPROVE THE GERMINATION AND EARLY SEEDLING GROWTH IN FINE RICE

M. Farooq, S.M.A. Basra, H. Rehman, M. Hussain and Y. Amanat

Department of Crop Physiology, University of Agriculture, Faisalabad-38040, Pakistan

(E-mail: farooqcp@gmail.com)

Pre-sowing salicylate seed treatments were employed in fine rice to explore the possibility of improvement in germination and early seedling growth. Fine rice (cv. Super-basmati) seeds were soaked in 10, 20 and 30 ppm aerated solutions of salicylate for 48 h at 28 ± 2 . Salicylate seed treatments resulted in earlier, synchronized and enhanced germination. Improvement in root length, leaf score, and seedling fresh and dry weight was also recorded due to pre-sowing salicylate seed treatments. However, lower shoot length from all the treatments and slower seedling dry weight from 10 ppm Salicylate seed treatments was noted. Seed treatments with 30 ppm salicylate solution was the most effective; however, root length and seedling dry weight was recorded 20 ppm salicylate treatment.

Keywords: Fine rice, salicylate, germination, seedling growth

INTRODUCTION

It has been estimated that half of the world's population subsists wholly or partially on rice. Rice has also emerged as a major export commodity contributing about 13% to the total valuable foreign exchange earning of Pakistan and accounts for 6.6% value added in agriculture and 1.6% in GDP in the country (Govt. of Pakistan, 2005).

Poor seedling establishment is one of the major yield limiting constraints both in transplanted and direct seeded rice especially under stressful conditions (Du and Toung, 2002). Use of high-quality seed better ensures seedlings, which emerge rapidly, tolerate adverse weather conditions, and resist diseases. Various pre-sowing seed treatments have been developed to invigorate the seeds (Basra *et al.*, 2002, 2003, 2004, 2005; Farooq *et al.*, 2004, 2005, 2006, 2006a). The purpose of these treatments is to shorten the time between planting and emergence (Farooq *et al.*, 2004, 2005, 2006) and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment (Senaratna *et al.*, 2000; Du and Toung, 2002; Shakirova *et al.*, 2003). Such treatments synchronize emergence, which leads to uniform stand and improved yield (Harris *et al.*, 2002; Du and Toung, 2002).

Improved seed performance has been achieved by incorporating plant growth regulators during pre-soaking, priming and other treatments in many crops (Jeong *et al.*, 1994) including rice (Miyoshi and Sato, 1997). Bhatt *et al.* (2000) found significant reduction of MGT and substantial improvement in germination when they used 100 ppm GA_3 pre-treatment in *Myrica esculenta*. Moreover, Qaderi and Cavers (2000) reported an increased germination rate by GA_3 of four

local populations of Scotch thistle. Miyoshi and Sato (1997) applied kinetin and gibberellins on dehusked seeds of indica and japonica rice to study their effects on the germination under aerobic and anaerobic conditions. They found stimulatory effects of gibberellin on the germination of indica and japonica rice seeds under both conditions, while, under anaerobic conditions, the responses of dehusked indica and japonica rice seeds to kinetin and gibberellin differed, being negative with kinetin and positive with gibberellin. Under aerobic conditions, the stimulatory effects of kinetin on germination of dehusked seeds were greater than those of gibberellin.

Salicylate is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants (Raskin, 1992). These include effects on ion uptake, membrane permeability, etc. (Barkosky and Einhelling, 1993). In addition, SA interacts with other signalling pathways including those regulated by jasmonic acid and ethylene (Szalai *et al.*, 2000, Ding and Wang, 2003). SA also induces an increase in the resistance of seedlings to osmotic stress (Borsani *et al.*, 2001), low or high temperature by activation of glutathione reductase and guaiacol peroxidase (Kang and Saltveit, 2002).

In an earlier study, Farooq *et al.* (2006) investigated the possibility of seed invigoration by seed treatments with salicylate and ascorbate in coarse and fine rice. Although, ascorbate was more effective in vigor enhancement, salicylate also improved the germination rate and seedling growth. The present experiment was therefore, planned to further explore the potential of salicylate seed treatments for vigor enhancement in fine rice.

MATERIALS AND METHODS

Seed materials

Seeds of fine rice cultivar (Super-Basmati) were obtained from Rice Research Institute, Kala Shah Kakoo, District Sheikhupura, Pakistan. The initial seed moisture contents were 8.34% (on dry weight basis).

Seed treatments

The seeds were soaked in 10, 20 and 30 ppm aerated solution of salicylic acid solution for 48 h at 28 ± 2 . The ratio of seed weight to solution volume was $1:5 \text{ (g mL}^{-1}\text{)}$ (Farooq *et al.*, 2006). After treating, seeds were dried near to their original weight, sealed in polythene bags and then stored in refrigerator at $7^\circ\text{C} \pm 1$ for further use.

Germination test

Seeds (15 in each) were placed in Petri dishes between layers of moist Whatman 45 at 27°C in an incubator. The completely randomized design with four replications was used. Germination was observed daily according to the AOSA method (AOSA, 1990). The time to get 50% germination (T_{50}) was calculated according to the following formulae of Coolbear *et al.*, (1984) modified by Farooq *et al.* (2005) as under:

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

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

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

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

Where n is the number of seeds, which were germinated on day D , and D is the number of days counted from the beginning of germination.

Energy of germination was recorded at 4th day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Farooq *et al.*, 2005).

Seedling emergence

Control and treated seeds were sown in 20×20 cm plastic trays (25 in each) having moist sand, replicated four times and were placed in growth chamber (Vindon, England) in completely randomized design. Day and night lengths were kept 15 and 9 h with 30°C

and 24°C temperatures, respectively (Farooq *et al.*, 2005). Relative humidity was maintained at 70%. Root and shoot length, and seedling fresh and dry weights were recorded 16 days after sowing. Number of leaves at harvest was designated as leaf score (Farooq *et al.*, 2006a).

RESULTS

Pre-sowing salicylic acid seed treatments significantly ($p < 0.05$) affected the germination and seedling growth in fine rice (Fig. 1-4).

All the salicylic acid seed treatments resulted in earlier germination compared with untreated seeds as shown by lower values of time to start germination (Fig. 1a), T_{50} (Fig. 1b) and MGT (Fig. 1c). Although, seeds treated with salicylic acid of different concentrations started germination on the same day (Fig. 1a) lowest T_{50} and MGT were recorded in seeds soaked in 30 ppm salicylic acid solution (Fig. 1b, 1c) (highly confusing). Similarly, all the salicylic acid seed treatments enhanced the energy of germination (GE) (Fig. 2a) and final germination percentage (FGP) (Fig. 2b) than that of untreated seeds. Maximum GE and FGP were recorded from the seeds soaked in 30 ppm salicylic acid solution (Fig. 2) followed by 20 ppm that was similar to the seeds soaked in 10 ppm salicylic acid solution.

Maximum shoot length was measured from the untreated seeds. All the salicylic acid seed treatments resulted in lower shoot length being minimum from the seeds soaked in 30 ppm salicylic acid solution (Fig. 3a). However, maximum root length was recorded from the seeds treated with 20 ppm salicylic acid (Fig. 3b) followed by that of 30 ppm. Minimum root length was noted from untreated seeds that was similar to that of seeds treated with 10 ppm salicylic acid (Fig. 3b). Salicylic acid seed treatments also resulted in statistically similar and higher leaf score than untreated seeds (Fig. 3c). All the seed treatments resulted in improved seedling fresh weight than that of untreated seeds (Fig. 4a) being maximum from the seeds soaked in 30 ppm salicylic acid solution followed by 20 ppm (Fig. 4a). However, minimum seedling dry weight was recorded from the seeds treated with 10 ppm salicylic acid solution followed by untreated ones (Fig. 4b), while other treatments resulted in higher seedling dry weight being maximum from the seeds treated with 20 ppm salicylic acid solution (Fig. 4b).

DISCUSSION

Pre-sowing salicylic acid seed treatments significantly ($p < 0.05$) improved the germination and early seedling growth in fine rice.

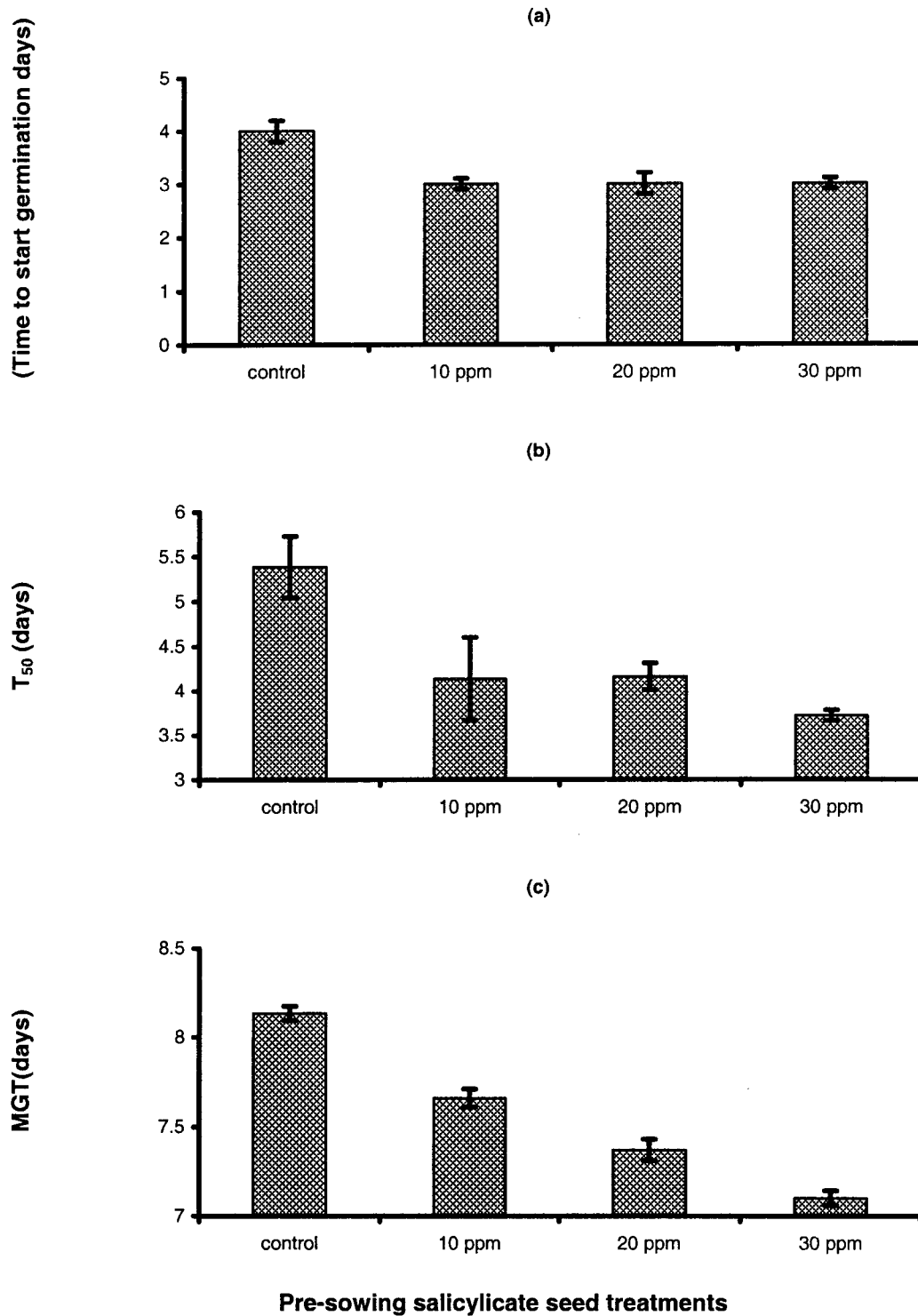


Fig. 1. Influence of Pre-sowing salicylic acid seed treatments on the (a) time to start germination, (b) time to 50% germination and (c) mean germination time

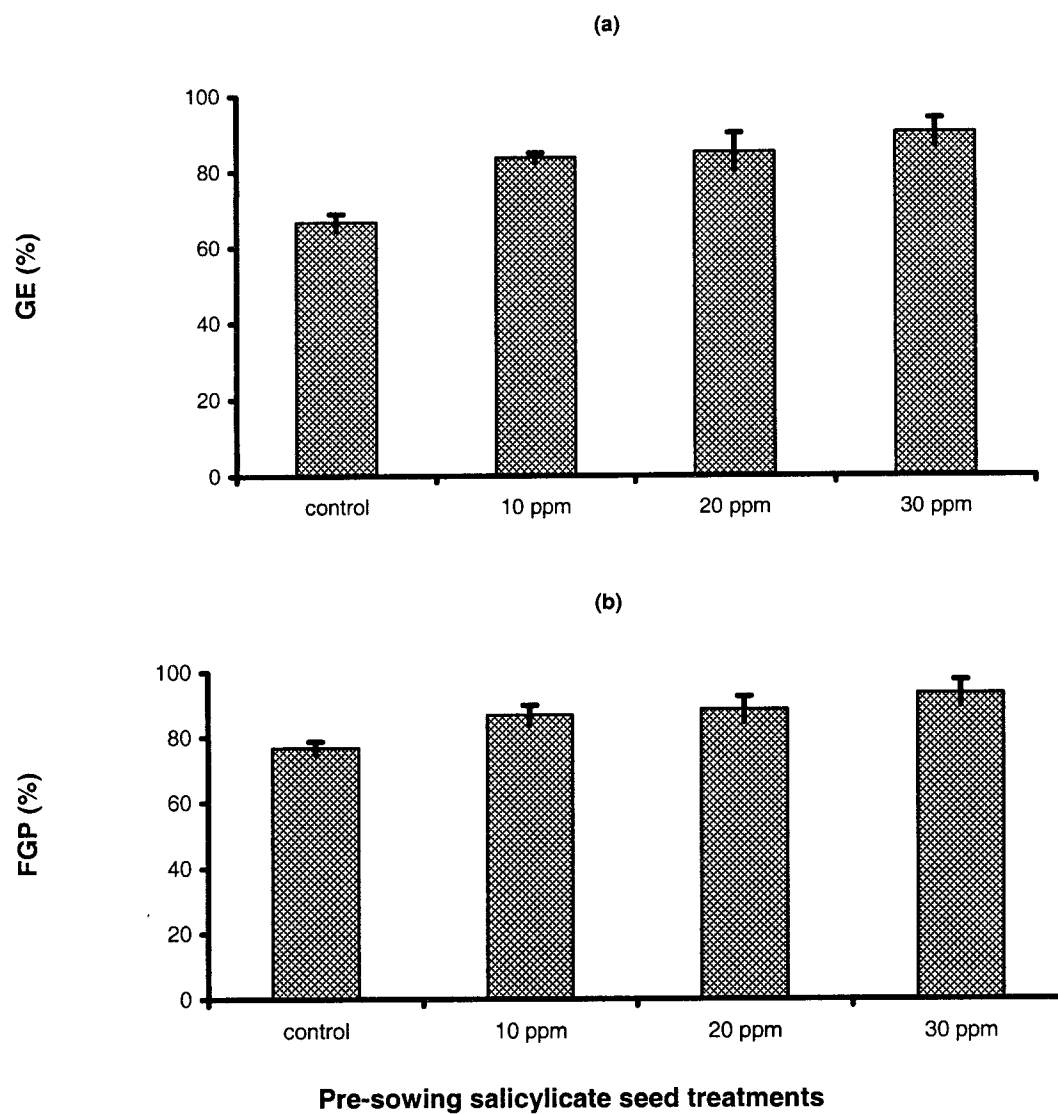


Fig. 2. Influence of Pre-sowing salicylic acid seed treatments on the (a) energy of germination and (b) final germination percentage

Rice seed invigoration

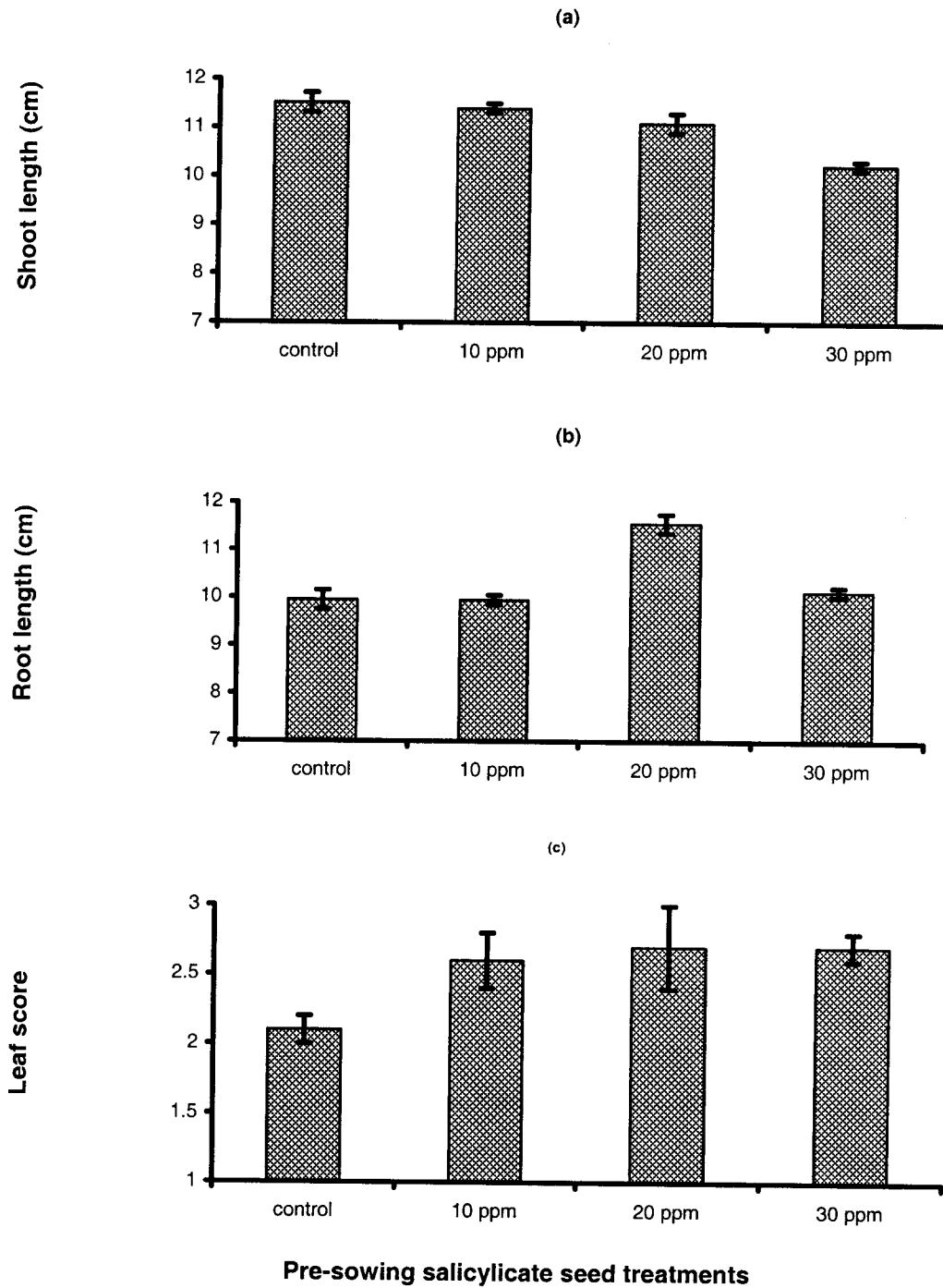


Fig. 3. Influence of Pre-sowing salicylic acid seed treatments on the (a) shoot, (b) root length and (c) leaf score

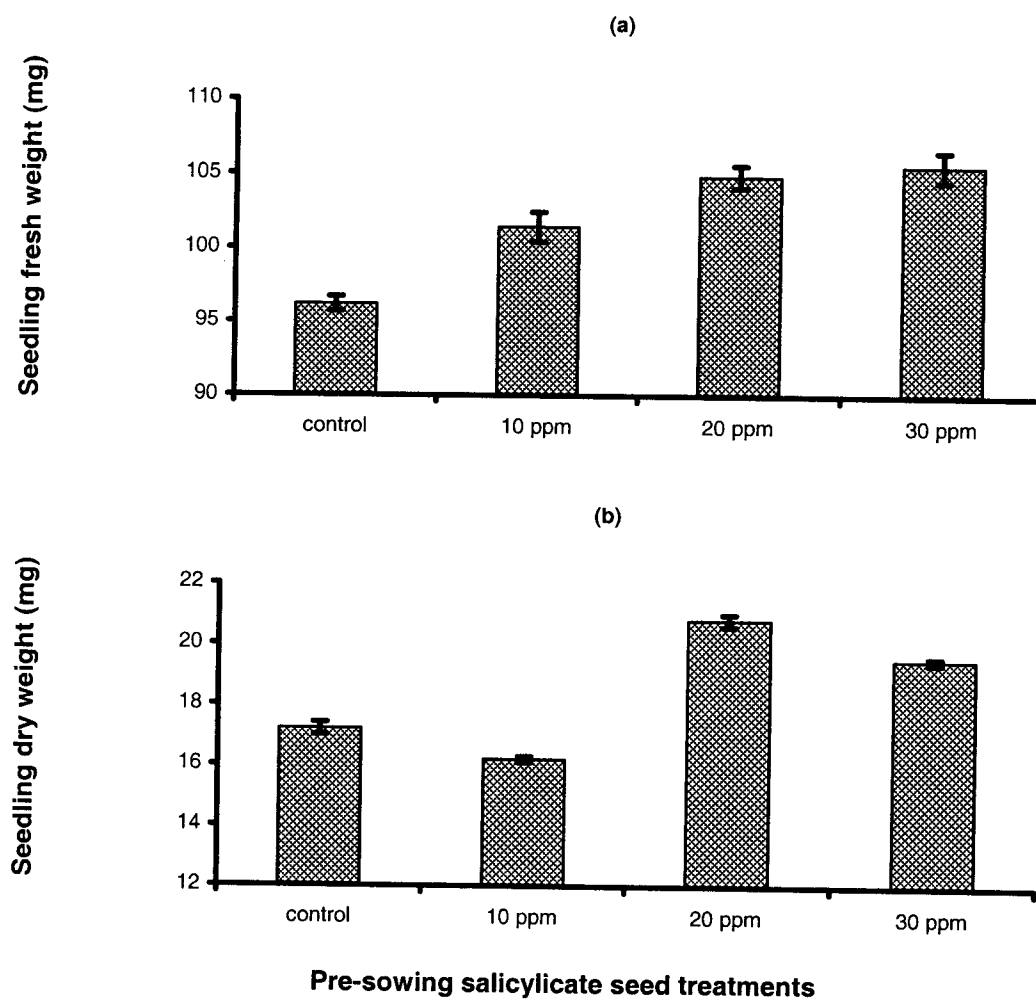


Fig. 4. Influence of Pre-sowing salicylic acid seed treatments on the (a) seedling fresh weight and (b) seedling dry weight

This study revealed that employing salicylate seed treatments could invigorate fine rice seeds. Earliest and most uniform germination was observed in seeds treated with 30 ppm salicylate as indicated by lesser time to start germination, MGT and T_{50} , and higher GE and FGP, root length, leaf score and seedling fresh and dry weight (Fig. 1-4). Lesser T_{50} and MGT indicated earlier and rapid germination while, higher GE express the power of germination i.e. germination spread over the time (Fig. 1-2). Earlier, Al-Hakimi and Hamada (2001) reported improved germination rate and percentage by ascorbate and sodium salicylate treatments in wheat. Increase in germination percentage after treatment might be the consequence of breakdown of dormancy as fresh seeds were used during the investigations. The earlier and synchronized germination might be attributed to increased metabolic activities in the treated seeds (Shakirova *et al.*, 2003; Basra *et al.*, 2005). Seed treatments not only improved the germination rate and time but also enhanced the seedling vigor as indicated by higher leaf score, root length and seedling fresh and dry weights (Fig. 3, 4). It is worth noting that salicylate seed treatments increased the root length but not the shoot length that might be explained by the fact that salicylate enhances the replication in root tips (Senaratna *et al.*, 2000; Shakirova *et al.*, 2003). Reduction in shoot length might be due to increased lateral growth as indicated by increased shoot weight which might have utilized the major part of food (Metwally *et al.*, 2003). This is confirmed by the fact that although shoot length was reduced (Fig. 3a), both seedling fresh and dry weights were increased due to salicylate treatments (Fig. 4). Improved seedling fresh and dry weights might be due to increased cell division within the apical meristem of seedling roots, which caused an increase in plant growth. Moreover, salicylate treatment maintains the IAA and cytokinin levels in the plant tissues, which enhances the cell division (Sakhabutdinova *et al.*, 2003). It may be concluded from the present studies that pre-sowing salicylate seed treatments improves the germination and early seedling growth in fine rice. However, seed soaking in 30 ppm salicylate solution was the most effective.

Acknowledgments

Authors acknowledge the Higher Education Commission, Government of Pakistan, for financial support of the present studies.

LITERATURE CITED

- Al-Hakimi, A.M.A. and A.M. Hamada. 2001. Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. *Biol. Plant*, 44, 253-261.
- Association of Official Seed Analysis (AOSA). 1990. Rules for testing seeds. *J. Seed Technol*, 12, 1-112.
- Barkosky, R.R. and F.A. Einhellig. 1993. Effects of salicylic acid on plant water relationship. *J. Chem. Ecol*, 19, 237-247.
- Basra, S.M.A., M. Farooq and A. Khaliq. 2003. Comparative study of pre-sowing seed enhancement treatments in fine rice (*Oryza sativa* L.). *Pak. J. Life and Soc. Sci*, 1, 5-9.
- Basra, S.M.A., M. Farooq, K. Hafeez and N. Ahmed. 2004. Osmohardening: A new technique for rice seed invigoration. *Inter. Rice Res. Notes*, 27, 74-75.
- Basra, S.M.A., M. Farooq, R. Tabassum and N. Ahmed. 2005. Physiological and biochemical aspects of seed vigor enhancement treatments in fine rice (*Oryza sativa* L.). *Seed Sci. Technol*, 33, 623-628.
- 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. *Pak. J. Arid Agric*, 5, 11-16.
- Bhatt, I.D., R.S. Rawal and U. Dhar. 2000. Improvement in seed germination of *Myrica esculenta* Buch: a high value tree species of Kumaun Himalaya, India. *Seed Sci. Technol*, 28: 597-605.
- Borsani, O., V. Valpuesta and M. Botella. 2001. Evidence for role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiol*. 126, 1024-1030.
- Coolbear, P., A. Francis and D. Grierson. 1984. The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *J. Exp. Bot*, 35, 1609-1617.
- Ding, C.K. and C. Wang. 2003. The dual effects of methyl salicylate on ripening and expression of ethylene biosynthetic genes in tomato fruit. *Plant Sci*, 164, 589-596.
- Du, L.V. and T.P. Tuong. 2002. Enhancing the performance of dry-seeded rice: effects of seed priming, seedling rate, and time of seedling. In: Direct seeding: Research strategies and opportunities. (eds). Pandey, S., M. Mortimer, L. Wade, T.P. Tuong, K. Lopes and B. Hardy. International Research Institute, Manila, Philippines, pp. 241-256.

- Ellis, R.A. and E.H. Roberts. 1981. The quantification of ageing and survival in orthodox seeds. *Seeds Sci. Technol*, 9, 373-409.
- Farooq, M., S.M.A. Basra, K. Hafeez and E.A. Warriach. 2004. The influence of high and low temperature treatments on the seed germination and seedling vigor of coarse and fine rice. *Inter. Rice Res. Notes*, 29, 69-71.
- Farooq, M., S.M.A. Basra, A. Wahid and M.B. Khan. 2006. Rice Seed Invigoration by Hormonal and Vitamin Priming. *Seed Sci. Technol*, 34, 775-780.
- Farooq, M., S.M.A. Basra, K. Hafeez and N. Ahmad. 2005. Thermal hardening: a new seed vigor enhancement tool in rice. *J. Integr. Plant Biol*, 47, 187-193.
- Farooq, M., S.M.A. Basra, H. Rehman and T. Mehmood. 2006a. Germination and Early Seedling Growth as affected by Pre-Sowing Ethanol seed Treatments in Fine Rice. *Int. J. Agric. & Biol.*, 8(1): 19-22.
- Govt. of Pakistan. 2005. Economic survey of Pakistan 2004-2005. Govt. of Pakistan, Finance Division, Economic Advisors Wing, Islamabad, Pakistan. Pp: 16-18.
- Harris, D., R.S. Tripathi and A. Joshi. 2002. On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice *In: Direct seeding: Research strategies and opportunities.* (eds.). Pandey, S., M. Mortimer, L. Wade, T.P. Tuong, K. Lopes and B. Hardy. International Research Institute, Manila, Philippines, pp: 231-240.
- 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. Kor. Soc. Hort. Sci.*, 35(5): 407-414.
- Kang, H.M. and M. Saltveit. 2002. Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. *Physiol. Plant*, 115, 571-576.
- Metwally, A., I. Finkemeier, M. Georgi and K.J. Dietz. 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiol*, 132-272-281.
- Miyoshi, K. and T. Sato. 1997. The effects of Kinetin and Gibberellin on the germination of dehusked seeds of indica and japonica rice (*Oryza sativa* L.) under anaerobic and aerobic conditions. *Ann. Bot.*, 80, 479-483.
- Qaderi, M.M. and P.B. Cavers. 2000. Interpopulation variation in germination responses of Scotch thistle, *Onopordum acanthium* L., to various concentrations of GA₃, KNO₃, and NaHCO₃. *Cand. J. Bot.*, 78, 1156-1163.
- Raskin, I. 1992. Role of salicylic acid in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*, 43, 439-463.
- Sakhabutdinova, A.R., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova. 2003. Salicylic acid e prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol*, Special Issue, 314-319.
- Senaratna, T., D. Touchell, E. Bumm and K. Sixon. 2000. Acetyl salicylic (Aspirin) and salicylic acid induce multiple stress tolerance in bean tomato plants. *Plant Growth Regul*, 30, 157-161.
- Shakirova, F.M., A.R. Sakhabutdinova, M.V. Bezrukova, R.A. Fatkhutdinova and D.R. Fatkhutdinova. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci*, 164, 317-322.
- Szalai, G., I. Tari, T. Janda, A. Pestenacz and E. Páldi. 2000. Effects of cold acclimation and salicylic acid on changes in ACC and MACC contents in maize during chilling. *Biol. Plant*, 43, 637-640.