

PRIMING WITH ETHANOL, ASCORBATE AND SALICYLATE ENHANCES THE GERMINATION AND EARLY SEEDLING GROWTH OF PEA (*Pisum Sativum* L.)

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Poor germination is one of the factors responsible for lower pea yield in Pakistan. Seed priming can be employed to solve the problem. This study was executed to investigate the possibility of improvement in germination and early seedling growth by seed priming in two pea cultivars climax and PF-400. For priming, seeds were soaked in aerated 0.5, 1 and 2% solutions of ethanol, and 1% solution each of ascorbate and salicylate for 12 h at 27 ± 2 . Both pea cultivars behaved similarly with slight differences. Priming in ethanol performed better than in ascorbate and salicylate. Priming in ethanol and salicylate solutions improved the germination rate and uniformity, and early seedling growth. Priming in 0.5% ethanol was at the top in improving time to 50% emergence, emergence energy, emergence percentage, root and shoot length, and seedling fresh and dry weights in both the cultivars. However, Priming in 2% ethanol performed the best in cultivar PF-400 for number of leaves and root length.

Keywords: Pea; seed priming; ethanol, ascorbate; salicylate; germination; seedling growth

INTRODUCTION

Peas (*Pisum sativum* L.) belong to family fabaceae and are self pollinated, cool season and hardy tendril plants. They are of great nutritional importance due to their high contents of protein, complex carbohydrates, dietary fiber, minerals, vitamins, and antioxidant compounds. Being short duration and nitrogen fixing can easily be adjusted in the existing cropping system and rotation. Among the factors responsible for low yield of pea is imbalance use of fertilizers, improper plant protection cover and sub optimum plant population. Sub-optimum plant population generally results from poor and erratic germination.

Seed priming has been reported to improve the germination rate and seedling growth in many field (Singh and Rao, 1993; Nagar *et al.*, 1998; Basra *et al.*, 2002, 2003, 2004, 2005; Farooq *et al.*, 2004, 2005, 2006; Hussain *et al.*, 2006) and vegetable crops (Heydecker and Coolbaer, 1977; Nerson *et al.*, 1985; Bradford, 1986; Nerson and Govers, 1986; Demir and Van de Venter, 1999; Demir and Oztokat, 2003; Farooq *et al.*, 2005a). It is a technique by which seeds are partially hydrated to a point where germination processes begin but radicle emergence does not occur (Heydecker and Coolbear, 1977; Bradford, 1986). Priming allows for some of the metabolic processes necessary for germination to occur without actual germination. Increased germination rate and uniformity have been attributed to metabolic repair during imbibition (Burgass and Powell, 1984; Bray *et al.*, 1989), a buildup of germination-enhancing metabolites (Coolbear *et al.*, 1980; Basra *et al.*, 2005), osmotic

adjustment (Bradford, 1986), and, for seeds that are not redried after treatment, a simple reduction in the lag time of imbibition (Heydecker and Coolbear, 1977; Bewley and Black, 1982; Brocklehurst and Dearman, 1983).

Salicylate (SA) 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). Salicylate 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.* (2006b) 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.

Ethanol has also been reported to have stimulatory effects on the germination of seeds of many plant species (Taylorson and Hendricks, 1979; Bewley and Black, 1982). In one study the dormancy problem in japonica rice was overcome by 0.5-5% ethanol treatment (Miyoshi and Sato, 1997).

Nerson and Govers (1986) subjected the seeds of muskmelon to priming and found that 2-3% solutions of $\text{KH}_2\text{PO}_4 + \text{KNO}_3$ (1:1) for 1-5 days significantly increased the germination rate, synchronization and percentage. However, dehydration of seeds following

treatments resulted in partial reversion of positive effects of priming. Osmotically primed tomato seeds also showed improved stand establishment, early seedling growth and yield, seedlings from primed seeds emerged earlier and more uniformly than seedlings from untreated seeds. Seedlings from primed seeds maintained greater mean plant dry weights, leaf areas and ground cover percentages than untreated seedlings throughout the pre-flowering period (Alvarado and Bradford, 1987).

One of the earliest attempts to improve pea (*Pisum sativum* L.) germination and seedling growth was done by Khan *et al.* (1978). He primed the pea seeds in 25, 35 and 50 % PEG solution over a period of 8 days and concluded that priming with polyethylene glycol not only shortened the time of germination but also improved the seedling growth rate. In another study, Sivritepe and Dourado (1995) primed pea seeds and observed that most of the benefits occurred during the first 3 days with PEG and during the first 5 days in distilled water. Priming treatments increased the final germination and decreased the mean germination time and the frequency of chromosomal aberrations, possibly due to the repair of some age induced damage.

Although, earlier, Khan *et al.* (1978) and Sivritepe and Dourado (1995) done very precious work on pea seed priming. No comprehensive study has been done including a wide range priming agents for pea seed priming. The present study was therefore aimed to evaluate the possibility of improving the germination and early seedling growth of pea by wide range seed priming treatments and to find out the most promising technique.

MATERIAL AND METHODS

Seed material

Seeds of pea cultivars Climax and PF-400 were obtained from Vegetable Research Institute, Ayyub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The initial seed moisture contents were 8.04% and 8.43% in Climax and PF-400, respectively.

Osmopriming

The seeds were soaked in aerated 0.5, 1, and 2% ethanol and 1% each of ascorbate and salicylate solutions for 12h h at 27 ± 2 . The ratio of seed weight to solution volume was 1:5 (g/mL) (Basra *et al.*, 2004).

Post treatment operations

After priming, seeds 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 seeds were then sealed in

polythene bags and stored in refrigerator at 5°C before further use.

Vigor evaluation

Control and treated seeds were sown in 5 kg plastic pots containing moist acid/water washed sand and placed in a net-house. The number of emerged seeds 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):

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

Where N is the final number of emerged seeds, and n_i and n_j the cumulative number of seeds 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 seeds, which were emerged on day D, and D is the number of days counted from the beginning of emergence.

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 (Farooq *et al.*, 2005). On the fifteen day after emergence, the seedlings were tested for vigor after carefully removing from the sand. Number of roots, 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

Seed priming significantly affected the germination and seedling growth in both pea cultivars. Both the cultivars responded similarly to different priming treatments with a little variation (Fig. 1-5).

In both pea cultivars, all the seed priming treatments resulted in earlier and uniform germination as shown by lower values of E_{50} and MET except ascorbate priming, which behaved similar to untreated seeds (Fig. 1). However, lowest E_{50} and MET were noted in seeds primed in 0.5% ethanol that was similar to that of seeds primed in 1% ethanol for E_{50} in both cultivars (Fig. 1a) and for MET in PF-400 (Fig. 1b). But

all the priming treatments improved the energy of emergence in both the cultivars than untreated seeds being maximum in seeds primed in 0.5% ethanol (Fig. 2a). All the priming treatments improved the final emergence in both cultivars except ascorbate priming, which behaved similar to untreated seeds (Fig. 2b). However, highest FEP was noted in seeds primed in 0.5% ethanol that was similar to that of seeds primed in 1% ethanol in climax (Fig. 2b).

Pea cultivars responded differently to priming treatments in case of number of leaves and number of roots (Fig. 3). In cultivar climax, ethanol treatments improved the number of leaves but ascorbate and salicylate treatments resulted in lower number of leaves compared with control (Fig. 3a). However, all the treatments improved the number of roots except ascorbate, which behaved similar to control in climax (Fig. 3b). In contrast, all the priming treatments improved the number of leaves and roots compared with control in cultivar PF-400 (Fig. 3a, 3b). Moreover in climax, maximum number of leaves and roots were noted from seeds primed in 0.5% and 1% ethanol, respectively (Fig. 3a, 3b); while in cultivar PF-400, seeds primed in 2% ethanol resulted in maximum leaves and roots, which was similar to ascorbate in case of number of roots (Fig. 3a, 3b). All the priming treatments improved the root length in both pea cultivars (Fig. 4a). But maximum root length was noted from seeds primed in 0.5% and 1% ethanol in cultivar climax and PF-400, respectively (Fig. 4a). In case of shoot length, seed priming treatments resulted in improvement in both pea cultivars except ascorbate for cultivar climax which behaved similar to control (Fig. 4b). However, in both cultivars, maximum shoot length was noted from seeds primed in 0.5% ethanol (Fig. 4a, 4b). In cultivar climax, ethanol treatments improved the seedling fresh weight but ascorbate and salicylate treatments resulted in lower seedling fresh weight compared with control (Fig. 5a). However, all the treatments improved the seedling fresh weight than that of control (Fig. 5a). In contrast, all the priming treatments improved seedling fresh and dry weight compared with control in cultivar PF-400 (Fig. 5a, 5b).

DISCUSSION

The study suggests that germination and early seedling growth of peas can be improved by employing seed priming techniques

Both pea cultivars behaved similarly with slight differences. Priming in ethanol was more effective than other treatments, however, ascorbate failed to improve the germination. Seed priming decreased the time to 50% emergence and mean emergence time and increased seedling emergence, seedling fresh and dry weight and root and shoot length. Seed priming

techniques not only resulted in earlier and more uniform emergence (as is clear from lower values of E_{50} and MET) but the emergence percentage and energy of emergence were also improved. The earlier and better-synchronized germination is associated with increased metabolic activities in the soaked seeds (Basra *et al.*, 2005). Earlier, Miyoshi and Sato (1997) had also reported effectiveness of ethanol treatments in dormancy breakdown in rice. Stimulation of the germination of caryopses by ethanol was reported in *Panicum dichotomiflorum* (Taylorson and Hendricks, 1979) and *Avena* spp. (Corbineau *et al.*, 1991). Two different mechanisms by which ethanol might break dormancy have been proposed; Taylorson and Hendricks (1979) suggested that the stimulatory effect of ethanol might involve modification of the properties of a membrane(s). Ethanol might also be involved metabolically in the stimulation of germination, as a respiratory substrate. It might accelerate germination by promoting the uptake of oxygen (Fidler, 1968; Adkins *et al.*, 1984) and increasing levels of fructose 2, 6-bisphosphate which has been suggested to stimulate glycolysis in dormant seeds of *Avena sativa* (Larondelle *et al.*, 1987). Improved seedling fresh and dry weights might be due to increased cell division within the apical meristem of seedling roots and shoot tips, which caused an increase in plant growth. Moreover, salicylic acid treatments maintain the IAA and cytokinin levels in the plant tissues, which enhanced the cell division (Sakhabutdinova *et al.*, 2003).

Poor performance of ascorbate is not in conformity with our earlier work on rice that concluded improved performance from rice seeds treated with ascorbate (Farooq *et al.*, 2006b). These results are also in contrast with the findings of Al-Hakimi and Hamada (2001) who reported improved germination rate and percentage by ascorbate treatments in wheat.

The interesting thing to note is that lower concentration of ethanol is more effective for improvement in germination and seedling growth which confirms our earlier study on rice (Farooq *et al.*, 2006b) which states that soaking rice seeds in ethanol solution having lower concentration enhance the germination rate, uniformity and final germination.

From the present study, it may be concluded that the seed priming with ethanol is more effective than in ascorbate and salicylate. Moreover, lower concentration of ethanol has more pronounced effect for improvement in germination and early seedling growth, while priming in ascorbate could not improve the performance rather it impaired the seedling emergence and growth.

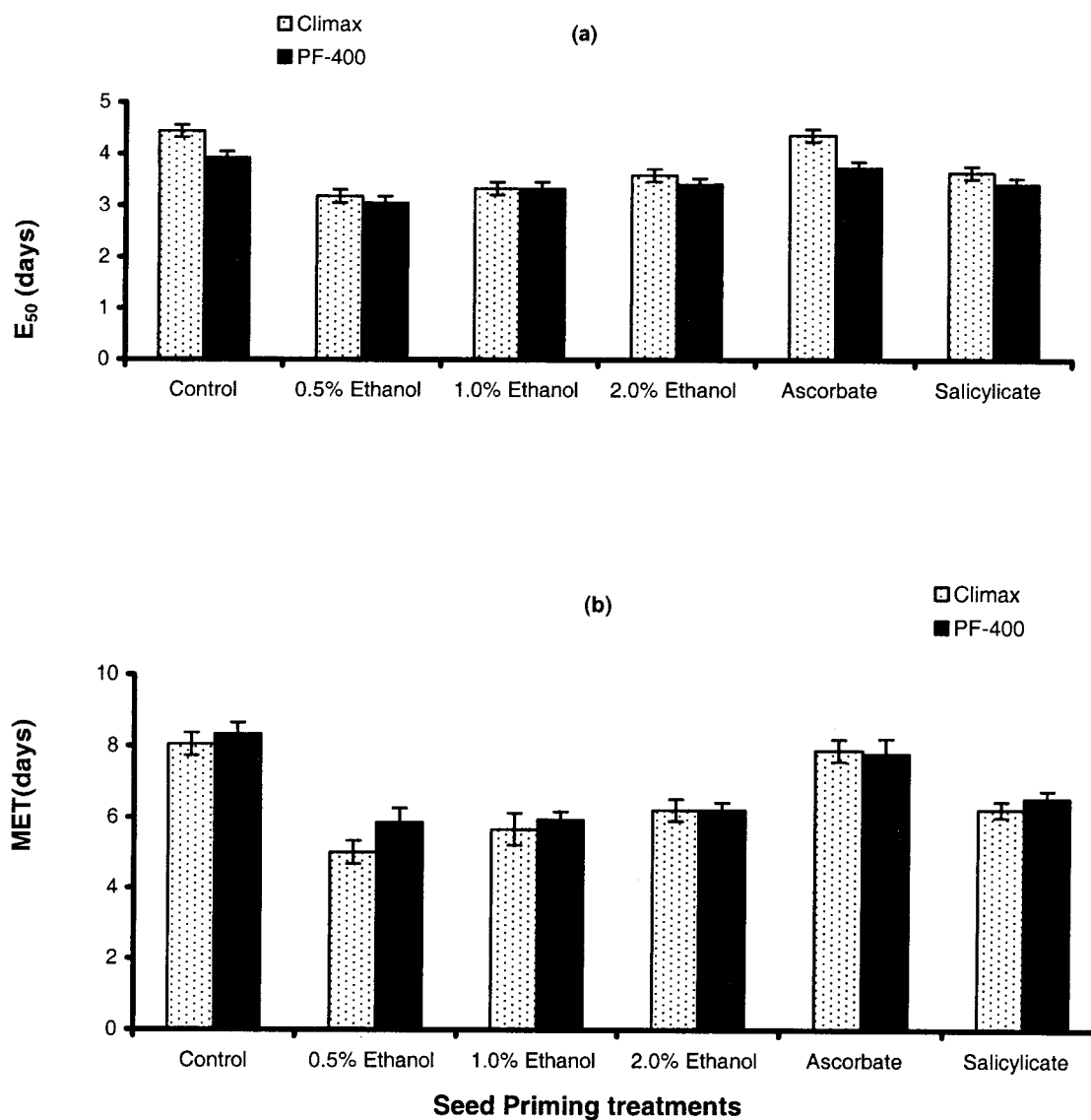


Fig. I. Influence of Seed priming treatments on the (a) Time to 50% emergence (E₅₀) and (b) mean emergence time (MET) in pea cultivars Climax and PF-400 \pm s.e.

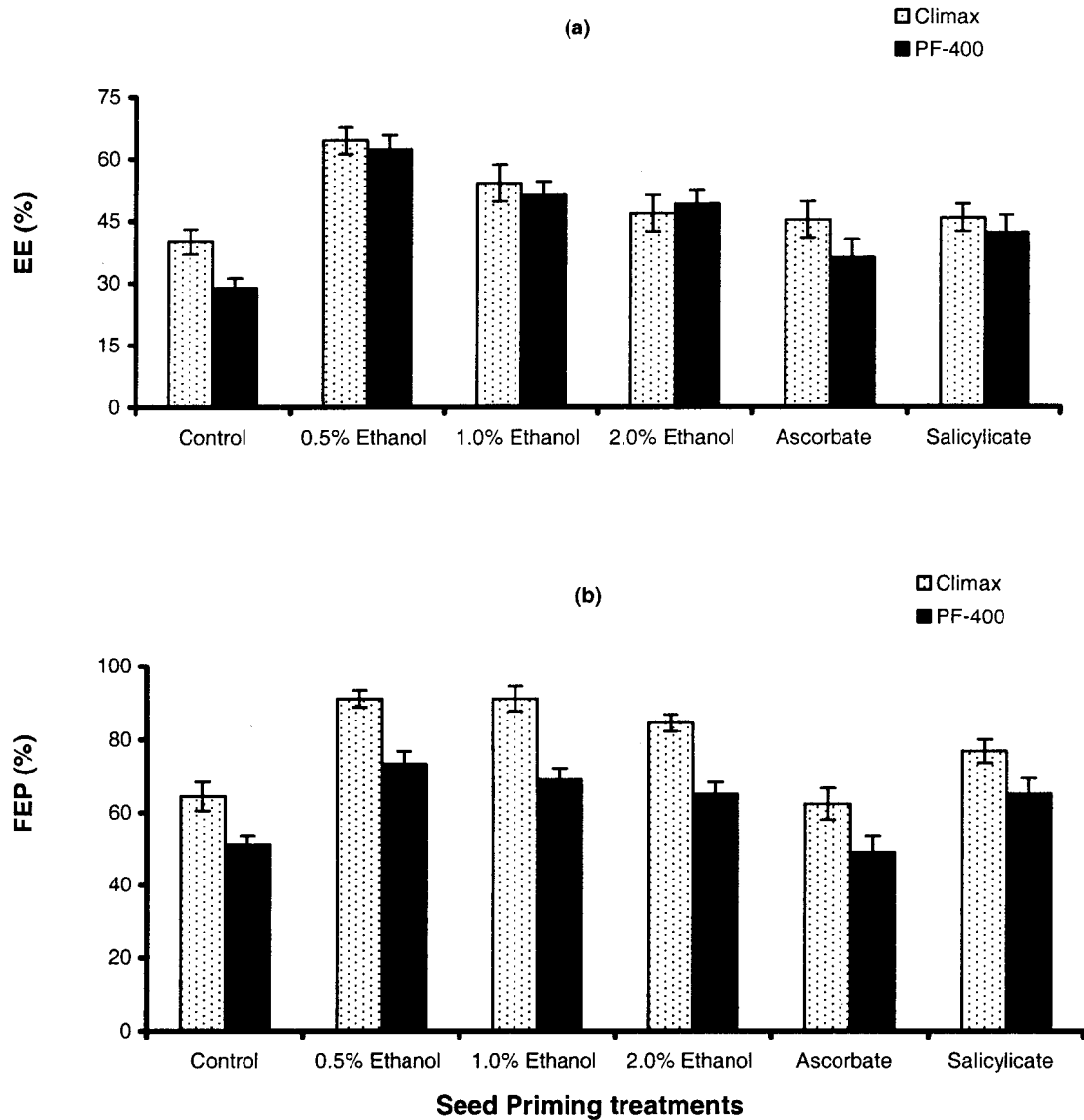


Fig. 2. Influence of Seed priming treatments on the on the (a) Emergence energy (EE) and (b) Final emergence percentage (FEP) in pea cultivars Climax and PF-400 \pm s.e. \pm s.e.

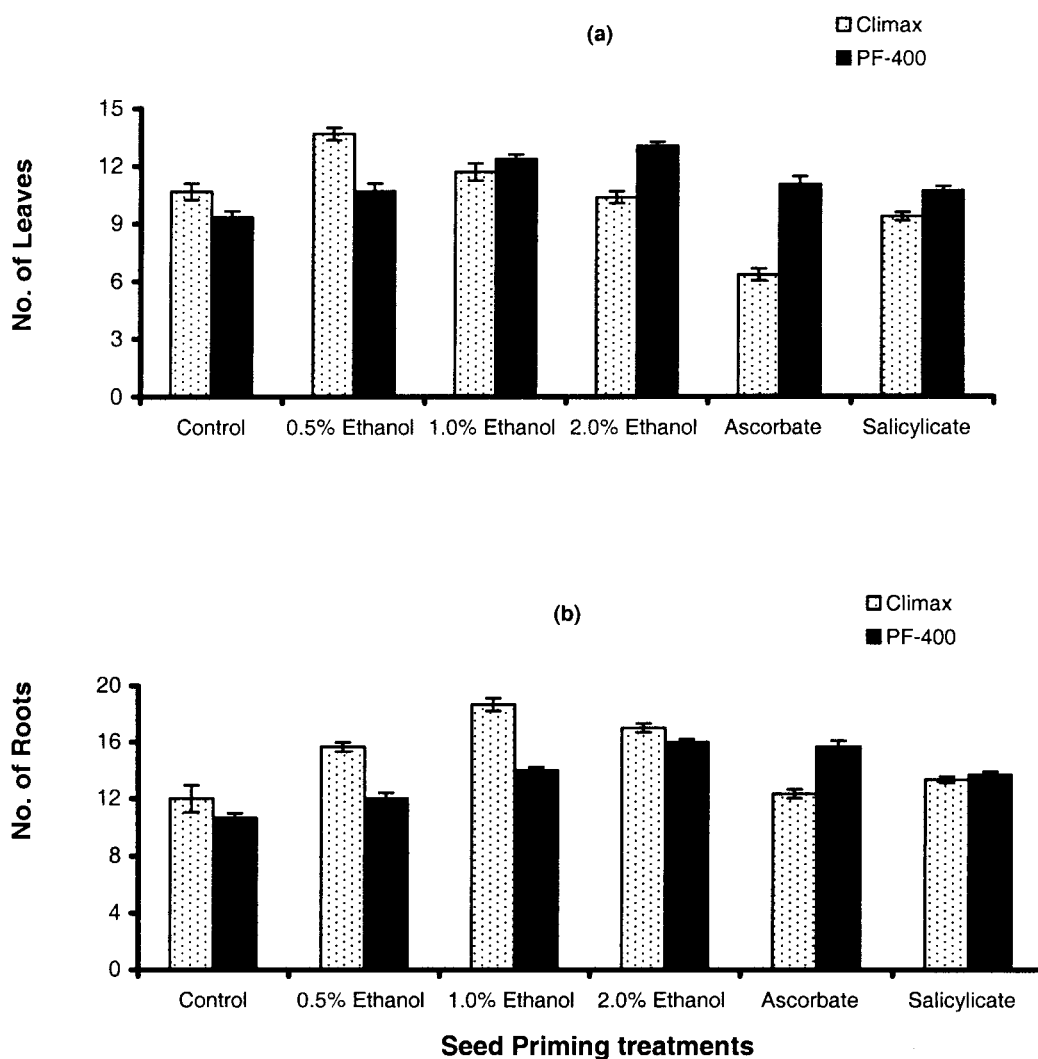


Fig. 3. Influence of Seed priming treatments on the on the (a) No. of leaves and (b) No. of roots in pea cultivars Climax and PF-400 \pm s.e.

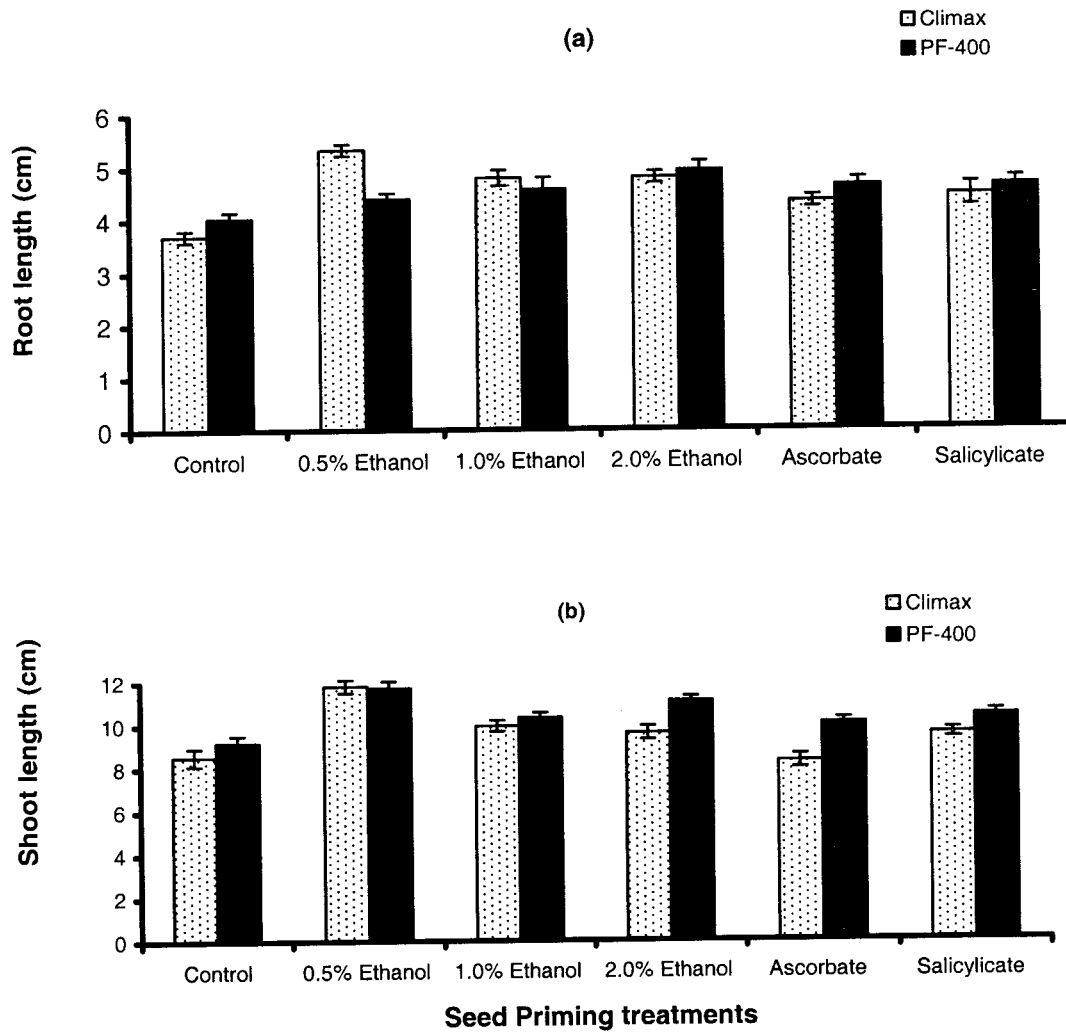


Fig. 4. Influence of Seed priming treatments on the on the (a) Root length and (b) Shoot length in pea cultivars Climax and PF-400 \pm s.e.

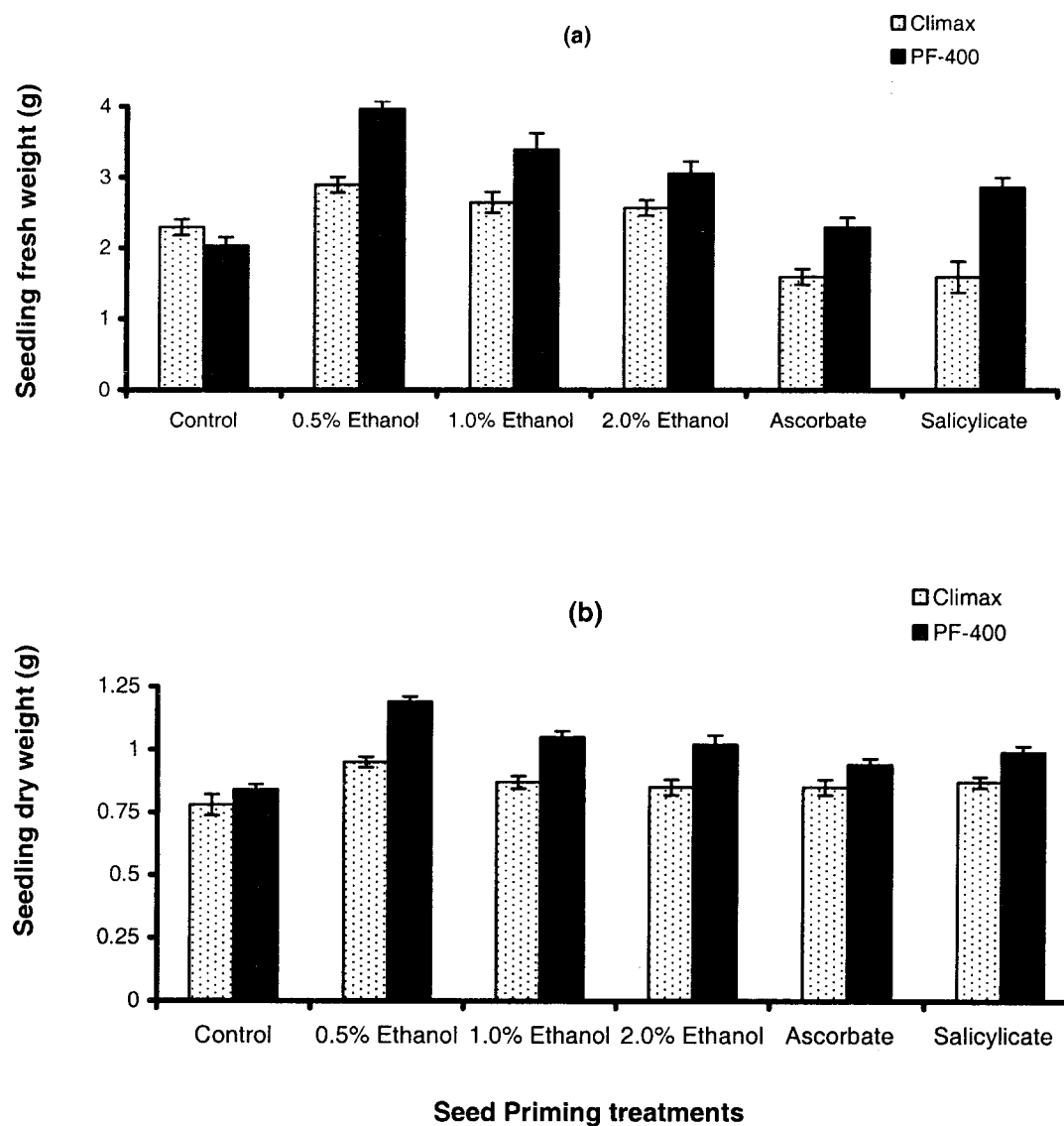


Fig. 5. Influence of Seed priming treatments on the on the (a) Seedling fresh weight and (b) Seedling dry weight in pea cultivars Climax and PF-400 \pm s.e.

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