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TOMATO SEED INVIGORATION WITH CYTOKININS

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ABSTRACT

The study was carried out to investigate the effects of hormonal priming on germination and seedling growth of tomato seeds. Priming was done by exposing seeds of two tomato cultivars 'Nagina' and 'Pakit' to aerated solutions of (Cytokinins) 10, 50 and 100 ppm BAP and kinetin for 24 h. The performance of primed and non primed seeds was evaluated during germination and emergence tests under controlled conditions by using completely randomized design with three replications. Seed priming with 10 ppm kinetin increased final germination percentage, germination index, shoot length and seedling fresh weight of both tomato cultivars as compared to all other presowing seed treatments including control. Seeds of both tomato cultivars primed with 10 ppm kinetin for 24 h significantly reduced the time taken to 50% emergence and mean emergence, increased final seedling emergence percentage and seedling growth. Results indicated that cytokinins priming with varying concentrations of BAP and kinetin improved germination potential and seedling establishment of both cultivars. Maximum improvement was recorded in seeds primed with 10 ppm kinetin. The better performance of primed seeds may be due to lower electrical conductivity (EC) of seed leachates.

Key words: Seed dormancy, cytokinins, electrical conductivity.

INTRODUCTION

Two major forms of physiological seed dormancy include embryo and seed coat dormancy. Germination commences with the uptake of water by imbibition of the dry seed, followed by embryo expansion. The rupture of endosperm is main germination-limiting factor in seeds of Solanaceae (e.g. tomato and tobacco). In case of endosperm-limited germination, weakening of the micropylar endosperm surrounding the radicle tip appears to be required for radicle protrusion, and is likely to involve cell-wall hydrolysis by hydrolytic enzymes. Major recent contributions to our understanding of endosperm-limited germination have come from research on tomato and Nicotiana species (Koornneef *et al*., 2002).

Endosperm rupture is the main limitation for germination of tomato seeds, weakening of the micropylar endosperm surrounding the radicle tip appears to be required for radicle protrusion and is likely to involve cell-wall hydrolysis by hydrolytic enzymes (Bewley, 1997). Due to this setback tomato production is badly affected all over the world. The physical strength of the endosperm, perisperm or seed coverings have been shown to restrict germination in cultivated crops like lettuce, tomato and cucumber (Dutta *et al*., 1994). Degradation of the endosperm in tomato is essential to initiate the germination.

Tomato is among the crops which are responsive to priming. The rationale of seed priming is to lessen the time between planting and emergence and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment and to synchronize emergence, which leads to uniform stand as well as improved yield. These priming treatments which enhance seed germination include hydropriming (Afzal *et al*. 2002) osmopriming (Rouhi, 2011), solid matrix priming (Ghassemi-Golezani *et al*., 2010) halopriming (Nawaz *et al*. 2011) and hormonal priming (Afzal *et al*., 2011). Cytokinins can also be used as priming agent; they are mainly involved in the breakdown of dormancy of some seeds, (Arteca, 1996). In this priming the seeds are soaked in aerated solution, which helps to invigorate the seed and facilitate the process of seed germination and seedling emergence evenly under adverse environmental conditions.

Many species are known where cytokinins alone break seed dormancy (Cohn and Butera, 1982). During the conditioning of parasitic Orobanche and Striga species and the release of lettuce thermoinhibition, cytokinins appear to contribute to the promotion of dormancy release and subsequent germination by enhancing ethylene biosynthesis (Saini *et al*., 1989; Matilla, 2000).

Cytokinins are present in developing seeds and accumulate predominantly in the liquid endosperm (Emery *et al*., 2000; Mok and Mok, 2001) which is a source of cytokinins needed for the promotion of cell division in the embryo. Primed seeds perform better in a wider range of temperatures and are less sensitive to oxygen deprivation (Corbineau *et al*., 1993) than unprimed ones. The favorable impact of priming has been associated with various, cellular, molecular and

biochemical events including synthesis of DNA and proteins (Bewley and Black, 1994). Priming can also help to increase enzyme activity and neutralize the effects of seed ageing.

This study was carried out to investigate the effect of seed priming with cytokinins on enhancing germination and seedling vigour of tomato seed and to explore effect of these priming treatments on different growth parameters of both tomato varieties.

MATERIALS AND METHODS

Seeds of two toamto cultivars i.e. Nagina and Pakit were collected from Ayub Agricultural Research Institute, Faisalabad, Pakistan. The initial seed moisture was 8.05% and 8.11% respectively (dry weight basis). Seeds were surface sterilized by dipping in sodium hypochlorite (5%) solution for 5 min and dried on filter paper. These surface sterilized seeds were soaked in aerated solution of 10, 50 and 100 ppm BAP and Kinetin for 24 h at 25 ^{o}C. Non primed seeds were considered as control. For hydropriming, seeds were soaked in distilled water. After respective priming treatment for specific period, seeds were washed with distilled water and dried at room temperature on filter paper in shade for 24 h.

Germination test: Twenty five seeds with three replicate per treatment were germinated in an incubator at 25°C under continuous fluorescent light (photosynthetic active photon flux density of 330 m mol m^{-2} S^{-1}) in a growth chamber (Vindon, England) in 9 cm Petri dishes on two layers of Whatman No.1 filter paper and moistened with 4 ml distilled water for fourteen days.

Time to 50% germination (T_{50}) was calculated according to the formulae of Coolbear *et al*. (1984). Mean germination time (MGT) was calculated according to Ellis and Roberts (1981). Germination index (GI) was calculated as described in the AOSA (1983). Energy of germination was recorded 4th day after planting. It is the percentage of germinating seeds 4th day after planting relative to the total number of seeds tested.

Emergence test: The primed and control (unprimed) seeds were sown in plastic trays (25 in each) having moist sand with three replications, placed in growth chamber (Vindon, England) maintained at 25°C under continuous fluorescent light for fourteen days. Emergence was recorded daily according to the seedling evaluation of the Handbook of Association of Official Seed Analysts (1983). Seedlings were harvested after two weeks and washed with deionized water after harvest. Afterwards they were separated into root and shoot for the determination of their fresh and dry weight. Dry weight was determined after oven drying the samples at 65°C for 48 h in oven.

Electrical conductivity of seed leachates: All priming treatments were helpful in reducing the electrical conductivity of seed leachates (Fig. 3). In general, the electrolyte leakage increased with increasing imbibition period including all treatments and control. After a longer period of imbibition from 1h to 24 h, all the priming treatments lowered down the electrolyte leakage in the seeds of both cultivars. After washing in distilled water, five seeds were weighed and soaked in 10 mL of distilled water at 25°C. Electrical conductivity of seed leachates was measured 0, 3, 6, 12 and 24 h after soaking using a conductivity meter (Twin Conductivity Meter, B-173, Horiba Ltd., Miyanohigashi, Kisshoin, Kyoto, Japan) and expressed as $\mu S\ cm^{-1}g^{-1}$.

Statistical analysis: The experiment was planned in completely randomized design with three replications; data recorded were pooled for statistical analysis using software Statistica to through analysis of variance. Duncan's multiple range test was used to compare the differences among treatment means.

RESULTS

Germination: Tomato seed priming with benzylaminopurine (BAP) and kinetin treatments had a significant ($P \leq 0.05$) effect on final germination percentage (FGP), mean germination time (MGT) and time taken to 50% germination of tomato during germination test. Priming with 10 ppm Kinetin showed maximum final germination in Nagina (87%) and Pakit (85%) which was statistically significant with hydropriming and control (Fig. 1). Minimum germination (50%) was recorded in unprimed seeds for Pakit. Hormonal priming treatments significantly affected the germination index, maximum germination index was recorded in hormonal priming with 10 ppm Kinetin followed by hormonal priming with 50 ppm Kinetin.

BAP and Kinetin showed a significant ($P \leq 0.05$) effect on reducing the mean germination time (Fig. 1). All of priming treatments except control took lesser time to germinate. Lowest MGT was recorded in seeds treated with 10 ppm Kinetin followed by 50 ppm Kinetin, while minimum MGT was recorded in unprimed seeds followed by hydropriming.

The time taken to 50% germination is a useful parameter in determining the vigour and uniformity of germination as the seeds which took lesser time to complete 50% germination are considered healthy which in turn resulted in better crop stand establishment.

Cytokinins priming treatments affected significantly ($P \leq 0.05$) fresh weight of seedling (Fig. 2). Maximum seedling fresh weight was recorded in seeds due to priming with 10 ppm Kinetin followed by priming with 50 ppm Kinetin, while the treatments with 50 ppm BAP and hydropriming were statistically at par.

Minimum seedling fresh weight was recorded in unprimed seed.

Dry weight of seedlings was also significantly (P≤0.05) affected by different cytokinins priming treatments. Seed treatments had a significant effect on seedlings dry weight (Fig. 2). Hormonal priming with 10 ppm Kinetin gave maximum dry weight followed by priming with 10 ppm BAP in improving seedlings dry weight as compared to other hormonal priming treatments, while minimum seedling dry weight was achieved in non-primed seeds.

Emergence: There was a significant (P ≤ 0.05) effect of pre-sowing seed treatments on final emergence percentage, mean emergence time and root length, shoot length and time taken to 50% emergence of tomato due to kinetin and BAP priming seed treatment. Maximum final emergence percentage (FEP) was recorded in case of seed priming with 10 ppm kinetin, followed by priming with 10 ppm BAP, which was statistically at par with hormonal priming with 50 ppm kinetin, while minimum FEP was depicted in unprimed seed.

BAP and Kinetin priming treatments showed a significant (P≤0.05) effect on mean emergence time (MET) (Table 1). All the primed seeds took lesser time to emerge as compared to the hydroprimed and unprimed seeds. Lowest MET was recorded in seeds primed with 10 ppm Kinetin followed by hormonal priming with 50 ppm Kinetin. Unprimed seeds took maximum time to emerge.

The time to 50 % emergence is a useful parameter in determining the vigour and uniformity of emergence as the seeds which took lesser time to complete 50% emergence, are considered healthy which in turn resulted in better crop stand establishment. All the priming treatments reduced the time taken to 50% emergence, maximum time taken to 50% emergence was depicted by unprimed seeds while minimum time to 50% emergence was shown by priming with 10 ppm Kinetin followed by priming with 50 ppm Kinetin (Table 1).

Emergence index indicates the power of a seed to emergence; this is a good indicator of seed vigour because higher the value of emergence index more will be the vigour of a seed. Highest emergence index was noted in seeds treated with 10 ppm Kinetin followed by priming with 10 ppm Kinetin, while minimum emergence index was recorded in unprimed seeds followed by hydropriming (Table 1). The cytokinins treatments showed significant effect on shoot length. The maximum shoot was found in the seed treated with 10 ppm Kinetin followed by priming with 50 ppm Kinetin. Minimum shoot length was depicted in case of hydropriming. Priming treatments significantly affected (P<0.05) fresh weight of seedling. Maximum seedling fresh weight was recorded in seeds due to priming with 10 ppm Kinetin followed by priming 50 ppm Kinetin, while minimum seedling fresh weight was recorded in hormonal priming with 50 ppm BAP. Seed treatments had a significant effect on seedlings dry weight (Table 1). Maximum seedlings dry weight was recorded in seed priming with 10 ppm Kinetin followed by priming with 50 ppm Kinetin in improving seedlings dry weight as compared to unprimed seed, while minimum seedling dry weight was achieved in priming with 50 ppm BAP.

Electrical conductivity of seed leachates: Pre-sowing cytokinins seed treatments were helpful in lessening electrolyte conductivity of seed leachates (Fig. 3). In general the electrolyte leakage increased with increasing imbibition period in all presowing see4d treatments and the control. After a longer period of imbibition ranging from l h to 24 h most of the priming treatments showed reduced electrolyte leakage except control. Maximum decrease in electrolyte leakage was induced by 10 ppm kinetin on all measuring periods. Seed priming with 10 ppm kinetin followed by 50 ppm kinetin was successful in decreasing electrolyte leakage.

DISCUSSION

Seed dormancy is an important developmental process allowing plants to endure especially unfavorable environmental conditions, such as drought or low temperature present in seeds of numerous plants. There are several factors which can help to overcome seed dormancy and that vary among species (Leon *et al.*, 2004). Cytokinins are one of the important groups of PGRs that control several important physiological activities in plants, such as photomorphogenesis. Our results showed that using cytokinins as seed priming agent could invigorate both tomato cultivars. Final germination percentage and time taken to 50% germination, germination index and mean germination time were improved in case of 10 ppm kinetin pretreatment as compared to unprimed (control).

Cytokinins are involved in a variety of processes in the growth and development of plants including cell division, root formation, leaf senescence, stomatal behaviour, and chloroplast development (Davies 1995; Brault and Maldiney 1999). The known interactions of cytokinins with other plant hormones as well as with environmental signals (Brault and Maldiney 1999), suggest that cytokinins modulate developmental processes in plants under harsh environmental conditions. The results of present investigation are in line with findings of Kepczynski *et al*. (1997) as kinetin is concerned in mobilization of storage reserves for utilization during germination.

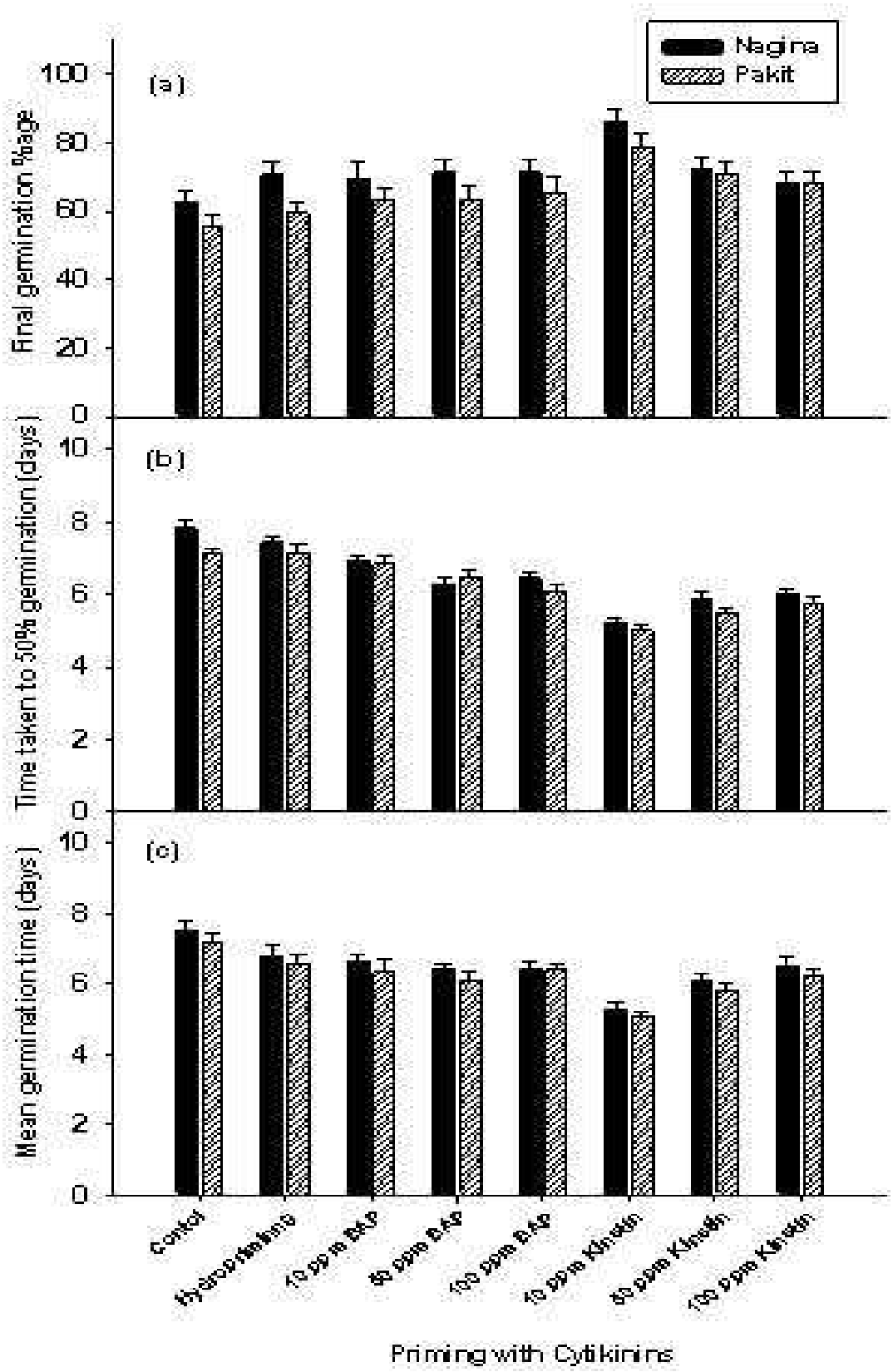

Fig. 1. Effect of different hormonal seed priming treatments on a) final germination % age, b) time taken to 50% germination and c) mean germination time of two tomato varieties Nagina and Pakit.

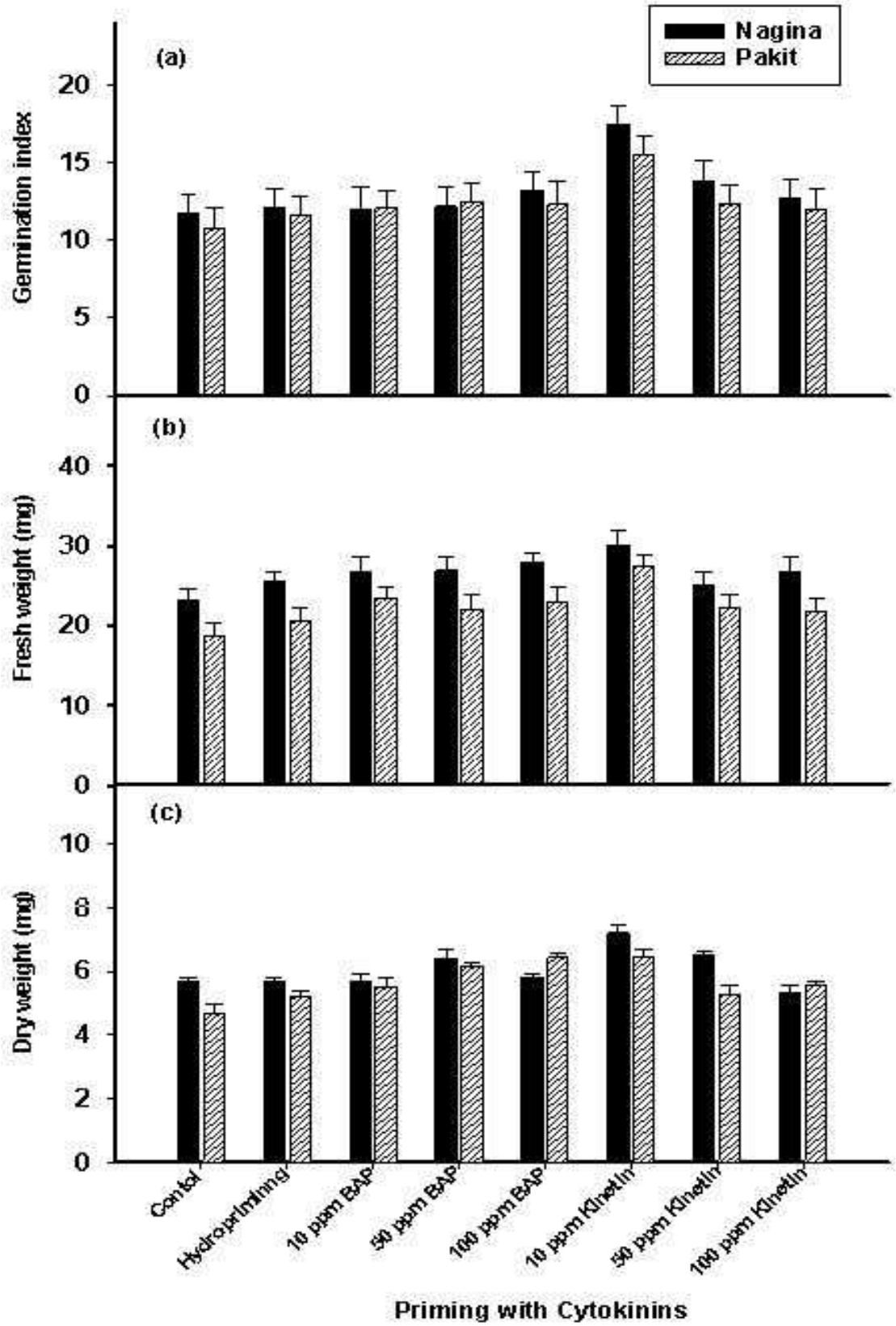

Fig. 2. Effect of different cytokinins seed priming treatments on a) germination index, b) seedling fresh weight and c) dry weight of two tomato varieties, Nagina and Pakit.

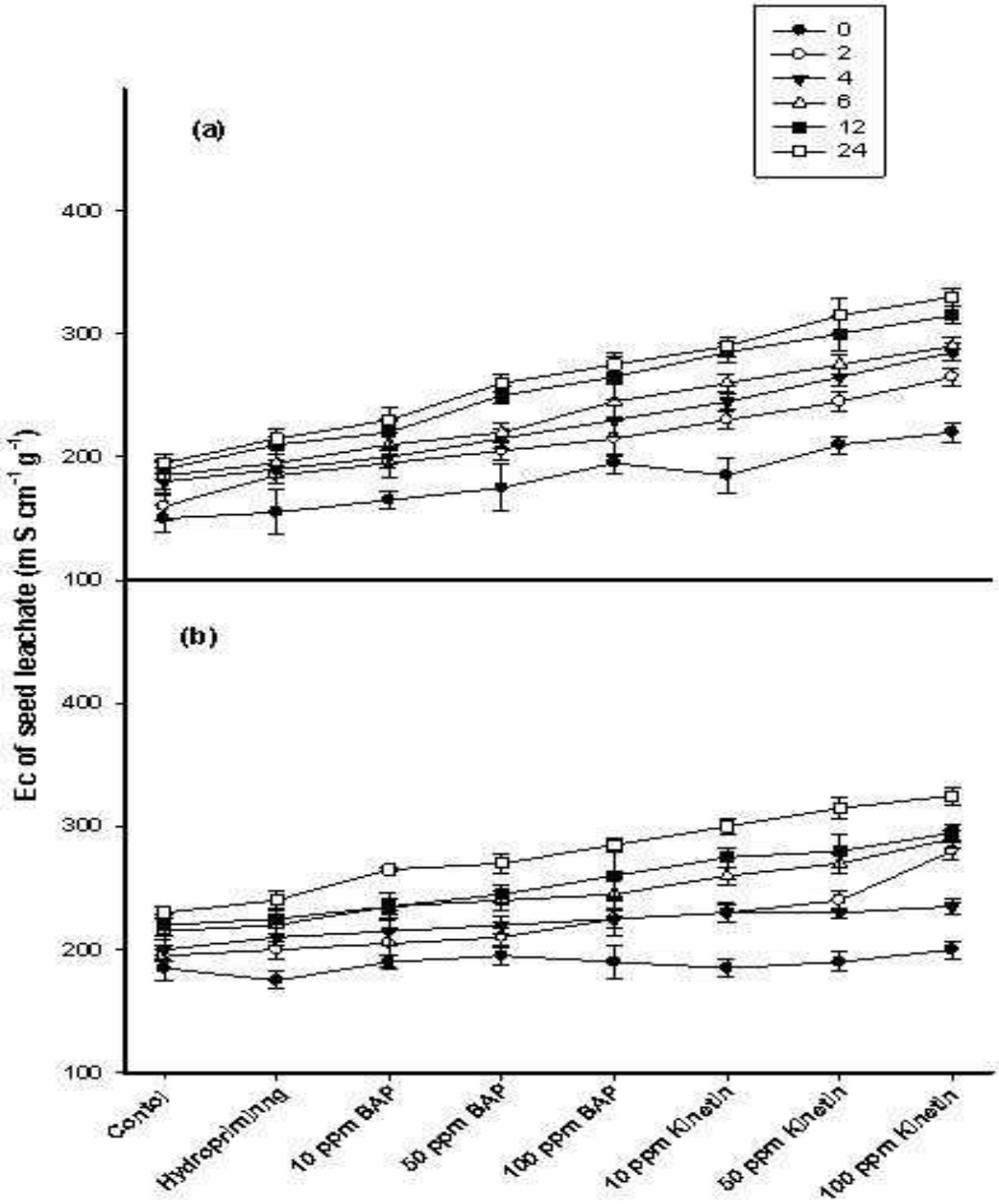

Fig. 3. Effect of Cytokinins priming treatments on electrical conductivity (EC) of seed leachates of two tomato varieties (a) Nagina and (b) Pakit.

Kinetin seed treatment at 10 ppm improved shoot lengths followed by 50 ppm kinetin while it was minimum in case of seed priming with 100 ppm BAP, which are in accordance with earlier work of Cary *et al*. (1995), that Benzyl amino purine (BAP) at high concentration inhibited shoot and root elongation. It has been reported earlier that cytokinins caused inhibition of the root and hypocotyls in *Arabidopsis thaliana* seedlings. Plant hormones are a group of organic substances which influence physiological processes mainly growth, differentiation and development. Cytokinins (CKs) and gibberellins (GAs) are found in actively dividing tissues of seeds; they are important in breaking dormancy after seed imbibition and allowing germination and growth of dormant embryos (Siobhan and McCourt, 2003). The fresh and dry weight of seedling were improved in case of seed priming with 10 ppm kinetin followed by 50 ppm kinetin and hydropriming which were statistically at par, while minimum seedling fresh and dry weights were recorded in case of 100 ppm BAP. These results are in accordance, with the studies of Patel and Saxena (1994) who reported that fresh and dry weight increased in seedlings raised from seeds treated with kinetin and GA_3 as compared to

seeds treated with NAA and Ethrel.

All the cytokinins priming treatments resulted in lower EC of seed leachates primarily due to improved membrane repair in treated seeds as reported by Rudrapal and Nakamura, (1988) for eggplant and radish and Farooq *et al.* (2005) for rice. The lesser EC values showed that cytokinins priming allowed the better membrane repair. Low EC induced by seed was accompanied with lower $T_{50,}$ MGT, and higher GI and GE in present studies. This suggests successful membrane and genetic repair and trigger of metabolic activities.

From this study it can be concluded that germination and seedling vigour may be enhanced by seed pretreatment with cytokinins in both the tomato cultivars Nagina and Pakit by dormancy breakdown. However kinetin with 10 ppm was more effective than all other cytokinins treatments.

Table 1. Effect of hormonal priming with cytokinins on germination and seedling vigour of tomato

	Priming	FEP	MET (days)	T50 (days)	Root length (cm)	Shoot length (cm)	Fresh wt. (mg)	Dry wt. (mg)
	Control	47.33 e	7.36 a	7.43 a	2.40 ab	4.93 a	22.53 c	6.23 c
	Hydropriming	52.00 d	7.08 bc	7.03 b	2.00 abc	4.76 a	25.76 b	7.16 b
	10 ppm BAP	66.00 bc	6.66 e	6.10 f	2.20 abc	4.40 ab	18.96 de	4.96 e
	50 ppm BAP	62.00 c	6.98 cd	6.40 de	1.96 abc	4.36 ab	21.50 c	5.36 d
Nagina	100 ppm BAP	54.00 d	7.30 ab	6.80 bc	1.33 d	4.26 ab	20.00 d	5.23 de
	10 ppm Kinetin	74.00 a	5.63 f	5.43 g	2.46 a	3.70 bc	27.13 a	7.73 a
	50 ppm Kinetin	68.00 b	6.56 e	6.20 ef	1.80 cd	2.93 cd	18.00 e	4.50 f
	100 ppm Kinetin	63.33 c	6.80 de	6.53 cd	1.86 bcd	2.76 d	16.33 f	4.06 g
	LSD at 0.05	**4.3560**	**0.2707**	**0.2691**	**0.5653**	**0.9097**	**1.1893**	**0.3789**
	Control	49.33 e	8.18 a	8.26 a	2.03 abc	4.36 a	24.60 bc	7.20 bc
	Hydropriming	52.00 de	7.66 b	8.16 a	2.26 a	4.33 a	26.43 b	8.23 ab
	10 ppm BAP	66.00 bc	6.46 c	7.16 b	2.03 abc	4.10 a	21.30 d	6.16 cd
	50 ppm BAP	62.00 c	7.48 b	6.40 cd	1.80 c	4.36 a	23.83 c	7.44 b
Pakit	100 ppm BAP	54.00 d	7.36 b	6.80 bcd	1.33 d	4.03 a	20.73 de	5.56 d
	10 ppm Kinetin	76.33 a	5.63 d	5.23 e	2.23 ab	4.73 a	30.16 a	9.19 a
	50 ppm Kinetin	68.00 b	6.56 c	6.20 d	1.86 bc	2.93 b	21.16 d	6.39 cd
	100 ppm Kinetin	62.00 c	6.80 c	6.90 bc	1.80 c	2.93 b	18.79 e	5.65 d
	LSD at 0.05	**4.0439**	**0.4931**	**0.6601**	**0.3822**	**0.7676**	**2.0993**	**1.0361**

Figures not sharing the same letters in a column differ significantly at p 0.05; FEP = Final emergence percentage, EI = Emergence index, MET = Mean emergence time, T50 = Time taken to 50% emergence.

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