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Priming of radish seeds with plant elicitors like SA, MeJA, BABA and KNO_3 improves seed quality, seedling quality and seed health

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Abstract
In the present experiment, radish seeds were subjected to priming treatment with different plant elicitors. There were 13 treatments with 4 plant elicitors and each of them was used as priming agents at 3 different concentrations *viz.* SA @ 25ppm, 50ppm and 75ppm, MeJA at 55 ppm, 110 ppm and 165 ppm, BABA at 250ppm, 500 ppm and 750ppm and KNO_3 at 1%, 2% and 3% and Untreated Control. The primed seeds were evaluated for seed quality, seedling quality and seed health parameters with paper towel method, blotter paper method and grow out test. The maximum germination (95.50%), SVI-I (2217.10) & SVI-II (1248.37) and other seed quality and seedling quality parameters were found significantly superior in potassium nitrate @ 2% which was observed at par when primed with salicylic acid @ 50ppm while priming with BABA @ 750 ppm and SA @ 75 ppm were most efficient in enhancing seed health by reducing infected seed percent, infected seedling percent and total seed mycoflora in radish seeds.

Keywords: Priming, SA, MeJA, BABA, KNO_3, Radish seeds

Introduction
Radish (*Raphanus sativus* L.) is an important root vegetable cultivated in tropical and temperate regions of the world. It is a diploid plant (2n=2x=18) belonging to family Crucifereae. Radish is preferred for its young fleshy enlarged edible roots consumed mainly as salad. Besides its use for culinary and as salad purpose it also possesses many medicinal values and its juice is highly recommended to treat jaundice patients.

Plant defense activators like salicylic acid (SA), Methyl Jasmonate (MeJA), β-amino butyric acid (BABA) and potassium nitrate (KNO_3) are agrochemicals that do not have direct toxic effect on pathogens and act by boosting plant defense mechanisms conferring resistance in plants against many pathogens. SA is recognized as an endogenous regulator of plant metabolism mainly involved in biotic and abiotic stress. SA application affects various physiological, biochemical and molecular processes in plants including antioxidative enzyme activities (Idrees *et al.*, 2011) [8]. It also plays an important role against pathogens, insect pests and abiotic stresses (Lu H, 2009) [11]. Jasmonic acid (JA) or jasmonates, are lipid-derived cyclopentanone compounds that occur ubiquitously and exclusively in the plant kingdom (Pirbalouti *et al.*, 2014) [14]. JA and related derivatives are known to mediate biotic and abiotic stress responses, in particular herbivory and wounding, and several developmental processes, such as seed germination, root growth, flowering, fruit ripening, and senescence (Delker *et al.*, 2006) [6]. In plant defense responses, JA acts as a wound hormone which is able to induce resistance pathways and defense gene expression. BABA is a non-protein amino acid which is proved to be a potent inducer of acquired resistance. It induces resistance in plants against a broad range of disease causing organisms including fungi, bacteria and viruses on a range of crop plants. BABA moves systemically and increase SA content in the leaf and induces different pathogenesis related (PR) proteins and also stimulates callose deposition around hypersensitive lesion and lignifications on tissue (Walters *et al.*, 2005) [20]. BABA-induced resistance (BABA-IR) mimics component of defense priming that are active during pathogen-induced systemic acquired resistance (SAR) and rhizobacteria-induced systemic resistance (ISR) (Van der Ent *et al.*, 2009) [19]. KNO_3 is a soluble source of two major essential plant nutrients. KNO_3 plays an important role in the adaptation of cells to abiotic stresses through their effect on water uptake, root growth, maintenance of turgour pressure and thereby can help in normal functioning of plants during pathogen attack (Bardhan *et al.*, 2007) [4]. Potassium takes part in protein synthesis, carbohydrate metabolism, and enzyme activation.

Potassium also plays a mitigating role in various abiotic stresses such as drought, salinity, metal toxicity, high or chilling temperatures etc. (Wang *et al.*, 2013) [21].

This experiment was conducted to find out the stimulating effect of priming with different concentrations of plant elicitors like salicylic acid, methyl jasmonate, β-amino butyric acid and potassium nitrate on seed quality, seedling quality and seed health of radish seeds.

Material and methods

The experiment was conducted in the laboratory of Department of Seed Science and Technology at Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during 2019 on radish seeds. In the experiment the variety used was Japanese White. The chemicals used were Salicylic acid, ($C_6H_4(OH)COOH$, Central Drug House Pvt. Ltd.), Methyl jasmonate ($C_{13}H_{20}O_3$, Sigma Aldrich), 3-amino butyric acid ($C_4H_9NO_2$, Sigma Aldrich), Potassium nitrate (KNO_3, Life Sciences Pvt. Ltd.) and sodium hypochlorite (NaOCl, Central Drug House Pvt. Ltd.)

There were thirteen treatments which are: T1: Seed priming with SA @ 25ppm, T_2: Seed priming with SA @ 50ppm, T_3: Seed priming with SA @ 75ppm, T_4: Seed priming with MeJA @ 55 ppm, T_5: Seed priming with MeJA @ 110 ppm, T_6: Seed priming with MeJA @ 165 ppm, T_7: Seed priming with BABA @ 250ppm, T_8: Seed priming with BABA @ 500ppm, T_9: Seed priming with BABA @ 750ppm, T_{10}: Seed priming with KNO_3 @ 1%, T_{11}: Seed priming with KNO_3 @ 2%, T_{12}: Seed priming with KNO_3 @ 3% and T_{13}: Control (Hydro priming). The experiment was laid out in Completely Randomized Design (CRD) with four replications of each treatment and each replication comprised of 100 seeds. Firstly the seeds were surface sterilized using 1% solution of sodium hypochlorite and then washed five times using distilled water and after that dried at room temperature under ceiling fan. The seeds were then primed by soaking them in solutions prepared using various concentrations of defense activators. Seeds were placed in beakers containing solution which was five times the volume of seeds. The seeds were soaked overnight at 25ºC. After that the seeds were rinsed in distilled water. Then the seeds were dried in shade for 24 hours (Shatpathy *et al.*, 2018) [18].

After priming the seeds were tested using paper towel method, blotter paper method and grow out test according to ISTA procedures (Anonymous, 1996) [3]. Observations were recorded for germination percent, Seedling length (cm), Seedling dry weight (mg), Seed Vigour Index (SVI - I) – Length, Seed Vigour Index (SVI - II) – Mass, Dead seeds (%), Speed of germination, Seed mycoflora, Percent infected seed, Seedling emergence (%), Speed of emergence, Normal seedlings (%), Abnormal seedlings (%) and Infected seedlings (%)

Statistical analysis

The data was statistically analyzed with the standard procedure as suggested by Gomez and Gomez (1984) [7]. The level of significance for different variables was tested at 5 per cent value of significance.

Results

The results from the experiment pointed that priming of radish seeds with plant elicitors had significant effect on all the parameter concerned with seed quality, seedling quality as well as seed health as compared to unprimed seeds or control.

The maximum germination percent (95.50%), speed of germination (87.17), seedling length (23.22 cm), seedling dry weight (13.07 mg), SVI-I (2,217.10) and SVI-II (1248.37) was recorded in seeds primed with KNO_3 @ 2% (T11) which was found to be statistically at par with seeds primed with SA @ 50ppm (T2) in which germination percent, speed of germination, seedling length, seedling dry weight, SVI-I and SVI-II were 94.75%, 84.51, 22.69, 12.87, 2149.66 and 1,218.99 respectively.

The maximum seedling quality parameters such as seedling emergence percent (78.25%), speed of emergence (30.14), normal seedling percent (78%) and the minimum ungerminated seed percent (21.75%) was observed in seeds primed with KNO_3 @ 2% (T11) which was statistically at par with SA @ 50ppm (T2) with 76.50% seedling emergence percent, 27.57 speed of emergence, 76.25% normal seedling percent, 23.50% ungerminated seed percent while minimum infected seedling percent (14.75%) was recorded in BABA @ 750ppm followed by 17% in SA @ 75ppm.

The effect on seed health parameters was also significant and minimum dead seed percent (3%) were observed in seeds primed with KNO_3 @ 2% while minimum infected seed percent (2.75%) and total seed mycoflora percent (18%), seeds infected with *Alternaria* sp. (16.75%), seeds infected with *Aspergillus* spp. (1.25%) were observed in seeds primed with BABA @ 750ppm which was followed by seed priming with SA @ 75ppm with 4% infected seed percent, 21% total seed mycoflora percent, 18.75% seeds infected with *Alternaria* sp. and 2% seeds infected with *Aspergillus* spp.

Table 1: Effect of seed priming with plant elicitors on seed quality parameters in radish seeds

Plant defense activator (conc.)		Seed quality parameters					
		Germination (%) **	Speed of germination	Seedling length (cm)	Seedling dry wt. (mg)	SVI-I (Length)	SVI-II (Mass)
T1	Salicylic acid (25ppm)	91.50 (9.62)	81.11	20.09	11.88	1,839.24	1,086.79
T2	Salicylic acid (50ppm)	94.75 (9.79)	84.51	22.69	12.87	2,149.66	1,218.99
T3	Salicylic acid (75ppm)	93.25 (9.71)	79.82	19.31	12.49	1,801.30	1,164.02
T4	Jasmonic acid (55ppm)	92.25 (9.66)	81.22	19.64	12.08	1,811.40	1,113.97
T5	Jasmonic acid (110ppm)	93.75 (9.73)	77.78	19.14	11.84	1,794.05	1,110.04
T6	Jasmonic acid (165ppm)	93.00 (9.70)	77.14	19.84	11.28	1,844.79	1,049.02
T7	Butyric acid (250ppm)	93.25 (9.71)	78.27	20.68	11.88	1,927.77	1,107.84
T8	Butyric acid (500ppm)	92.50 (9.67)	81.02	19.01	12.23	1,758.48	1,131.19
T9	Butyric acid (750ppm)	89.25 (9.50)	74.78	17.49	11.18	1,559.49	997.37
T10	Potassium nitrate (1%)	94.25 (9.76)	83.26	20.07	12.43	1,890.49	1,171.17
T11	Potassium nitrate (2%)	95.50 (9.82)	87.17	23.22	13.07	2,217.10	1,248.37
T12	Potassium nitrate (3%)	91.25 (9.60)	80.04	19.47	12.81	1,777.69	1,168.52
T13	Control	90.00 (9.54)	80.16	19.94	11.05	1794.27	993.97
C.D.(0.05)		0.10	3.56	1.27	0.40	121.125	42.96

** Figures in parentheses are square root transformed value

Table 2: Effect of seed priming with plant elicitors on seedling quality parameters in radish using grow out test

Plant defense activator (conc.)		Seed quality parameters				
		Seedling emergence (%)*	Speed of emergence	Normal Seedlings (%) **	Infected seedlings (%)*	Ungerminated seeds
T1	Salicylic acid (25ppm)	71.00 (57.40)	23.73	70.75 (57.25)	23.25 (4.92)	29.00 (32.56)
T2	Salicylic acid (50ppm)	76.50 (61.00)	27.57	76.25 (60.83)	22.25 (4.82)	23.50 (28.96)
T3	Salicylic acid (75ppm)	67.25 (55.08)	20.90	66.75 (54.78)	17.00 (4.24)	32.75 (34.88)
T4	Jasmonic acid (55ppm)	70.50 (57.09)	19.75	70.25 (56.93)	30.25 (5.59)	29.50 (32.87)
T5	Jasmonic acid (110ppm)	68.75 (56.01)	21.45	68.50 (55.85)	25.75 (5.17)	31.25 (33.96)
T6	Jasmonic acid (165ppm)	67.75 (55.39)	24.43	67.50 (55.24)	23.50 (4.95)	32.25 (34.58)
T7	Butyric acid (250ppm)	71.75 (57.88)	22.95	71.50 (57.72)	20.00 (4.58)	28.25 (32.08)
T8	Butyric acid (500ppm)	74.50 (59.65)	26.46	74.25 (59.49)	18.75 (4.44)	25.50 (30.31)
T9	Butyric acid (750ppm)	65.50 (54.02)	17.52	65.00 (53.72)	14.75 (3.97)	34.50 (35.95)
T10	Potassium nitrate (1%)	73.75 (59.17)	27.88	73.75 (59.17)	24.50 (5.05)	26.25 (30.80)
T11	Potassium nitrate (2%)	78.25 (62.19)	30.14	78.00 (62.01)	23.25 (4.92)	21.75 (27.78)
T12	Potassium nitrate (3%)	67.25 (55.07)	23.76	67.00 (54.92)	17.75 (4.33)	32.75 (34.89)
T13	Control	68.25 (55.77)	17.54	67.75 (55.44)	36.50 (6.12)	31.75 (34.20)
C.D. (0.05)		2.50	3.14	2.41	0.24	2.00

*Figures in parentheses are angular transformed value
** Figures in parentheses are square root transformed value

Table 3: Effect of seed priming with plant elicitors on seed health parameters in radish using paper towel and blotter paper method

Plant defense activator (conc.)		Seed Health Parameters				
		Dead Seed (%) **	Infected seed (%) **	Seed Mycoflora		
				Alternaria sp.**	*Aspergillus* spp.**	Total*
T1	SA (25ppm)	5.50 (2.54)	5.75 (2.59)	22.25 (4.82)	3.00 (1.99)	25.75 (30.47)
T2	SA (50ppm)	3.00 (1.99)	5.25 (2.50)	20.50 (4.64)	2.25 (1.80)	23.25 (28.80)
T3	SA (75ppm)	4.75 (2.39)	4.00 (2.23)	18.75 (4.44)	2.00 (1.72)	21.00 (27.25)
T4	JA (55ppm)	5.00 (2.45)	6.50 (2.74)	23.25 (4.92)	6.00 (2.64)	30.00 (33.19)
T5	JA (110ppm)	4.75 (2.39)	6.00 (2.64)	22.75 (4.87)	4.25 (2.28)	27.75 (31.77)
T6	JA (165ppm)	5.25 (2.50)	5.25 (2.50)	20.50 (4.64)	2.75 (1.93)	23.75 (29.15)
T7	BABA (250ppm)	5.00 (2.44)	4.25 (2.29)	21.75 (4.77)	3.25 (2.06)	25.75 (30.47)
T8	BABA (500ppm)	5.50 (2.54)	3.75 (2.17)	19.50 (4.53)	2.50 (1.87)	22.00 (27.96)
T9	BABA (750ppm)	6.50 (2.72)	2.75 (1.93)	16.75 (4.21)	1.25 (1.49)	18.00 (25.09)
T10	KNO_3 (1%)	4.50 (2.33)	6.00 (2.64)	22.75 (4.87)	5.75 (2.59)	29.00 (32.57)
T11	KNO_3 (2%)	3.00 (1.99)	5.50 (2.55)	20.75 (4.66)	5.00 (2.45)	26.00 (30.64)
T12	KNO_3 (3%)	5.50(2.54)	5.00 (2.45)	19.50 (4.53)	3.25 (2.06)	22.75 (28.47)
T13	Control	8.50 (3.08)	6.75 (2.78)	27.00 (5.29)	6.75 (2.78)	35.00(36.26)
C.D. (0.05)		0.34	0.23	0.22	0.26	1.39

*Figures in parentheses are angular transformed value
** Figures in parentheses are square root transformed value

Discussion

The increase in seed quality parameters can be attributed to more nitrogen and potassium accumulation in seeds treated with KNO_3 (Alevarado and Bradford, 1988). Kattimani *et al.* (1999) [10] stated that priming resulted in liberation of enzymes, thus rapidly increasing in the production of soluble food nutrients, the whole system is already in motion so that when the seeds are sown developmental processes go on more rapidly than in case of non-primed seeds. Thus when KNO_3 is used in priming solution the ready form of nitrogen and potassium is readily available to the embryo. These mechanisms might have played a role in increasing the germination percentage, speed of germination, seedling length, seedling dry weight, seed vigour index, seedling emergence percentage, higher speed of emergence and higher normal seedling percentage by activating the cellular metabolism and reducing cellular damage of seeds after priming with 2% of KNO_3 in the present investigation. McDonald (2000) already proved that seed priming permits early DNA replication, increase RNA and protein synthesis, enhances embryo growth, repairs deteriorated seed parts and reduces leakage of metabolites which may result in less percentage of dead and ungerminated seeds.

The statistically equivalent effect of SA @ 50ppm is because of a strong up-regulation of translation initiation and elongation factors, proteases, and two subunits of the 20S proteasome which were observed by SA treatment (Rajjou *et al*., 2006) [15], which supports the hypothesis that SA improves seed germination by promoting the synthesis of proteins that are essential for germination, and the mobilization or degradation of seed proteins accumulated during seed maturation. In addition, the biosynthesis of several enzymes involved in metabolic pathways such as the glyoxylate cycle, the pentose phosphate pathway, glycolysis, and gluconeogenesis is also strongly activated by SA, suggesting that SA promotes the release from a quiescence state to the establishment of a vigorous seedling (Rajjou *et al*., 2006) [15]. These are the possible mechanisms which might have resulted in increase of various seed quality parameters like seed germination, speed of germination, seedling length, seedling dry weight, seed vigour index, seedling emergence percentage, speed of emergence, normal seedling percentage and reduction in percentage of ungerminated seeds. Seed priming with SA @ 50ppm (T2) also reduced dead seed percentage in current study. Due to stress, accumulation of ROS takes place which cause extensive damage including lipid peroxidation, chlorophyll breakdown, loss of photosynthetic activity and membrane integrity, as well as electrolyte leakage (Ananieva *et al*., 2002) [2]. Pre-treatment of seedlings with SA reduces H_2O_2 production, lipid

peroxidation, and electrolyte leakage (Ananieva *et al*., 2002) [2]. The observed protection conferred by SA could be the result of a very rapid detoxification of ROS. The same mechanism might have enacted in the present investigation which might have resulted in reduction of damage to seed membrane by ROS, which further reduced the percentage of dead seeds in this study after priming of seeds with SA.

Seed priming with SA @ 75ppm (T3) reduced infected seedlings (%). This is because the activity of phenolic enzymes namely, peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) was found to increase after SA treatments (Sangha *et al*., 2007) [16]. Polyphenol oxidase and phenylalanine ammonia lyase aid the synthesis of quinones from phenols and biosynthesis of phenylpropanoid units for synthesis of lignins and flavonols, respectively. These enzymes also have a role in restricting the spread of the pathogen. Presence of phenols and their oxidative products in plant tissue is considered toxic to growth and development of the pathogens (Mandavia *et al*., 2000) [12]. This mechanism would have played the role in reducing the seed health parameters after priming with SA under present investigation.

Jensen *et al*. (1998) [9] explained that the mechanism by which BABA acts as an inducer of resistance could be either by its diffusion through the microscopic ruptures caused by imbibition of seeds or by initiation of interaction upon seed germination and diffusion into coleoptiles and radicals by non differentiated tissues of the germinating embryos, nevertheless another possible mechanism is interference of the soluble substance with the normal amino acid and/or protein synthesis that affects the metabolism of the host rather than the fungus, thus making the interaction incompatible (Cohen *et al*., 1999) [5]. These mechanisms might have played their role under the present investigation which reduced the infected seed (%) and seed mycoflora. BABA reduced seed infection significantly and similar results observed after treating seeds of pearl millet treated with 50mM BABA that reduced downy mildew disease incidence by 75% in seedlings raised from such seeds (Shailasree *et al*., 2001) [17].

Conclusion

Priming of radish seeds with plant elicitors like potassium nitrate @ 2% and salicylic acid @ 50 ppm were observed as effective treatments for enhancing the seed and seedling quality parameters like germination percentage, seedling length (cm), seedling dry weight (mg), seed vigour index, dead seed percentage, speed of germination, seedling emergence percentage, speed of emergence, normal seedlings percentage. While priming of radish seeds with BABA @ 750 ppm and SA @ 75ppm was most effective in reducing the seed mycoflora, percent infected seed and infected seedlings percentage.

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