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Effect of pre-sowing growth regulator treatments on germination and seedling growth of garden rue (*Ruta graveolens* Linn.)

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ABSTRACT : The seeds of garden rue were subjected to pre- sowing treatments with gibberellic acid (50, 100, 200, 300 and 400ppm), benzyl adenine (25, 50, 100 and 200,ppm), ethrel (100,200,300,400 ppm) and distilled water as control for three hours. Germination and seedling growth parameters at definite intervals were recorded to find out the effect of these pre-treatments on germination of garden rue. The maximum germination percentage (89.33), plant height (12.30 and 16.33 cm, at 30 and 60 days after sowing (DAS), respectively), collar diameter(3.60 and 4.53mm, at 30 and 60 DAS, respectively), root length (9.50 cm), root number (25.67), fresh weight of shoots(5.81g) and vigour index I (2299.47) were recorded in the seeds treated with GA_3 at 400 ppm. Whereas, GA_3 300 ppm recorded the highest rate of germination(10.27), compound leaves (6.60 and 10.27 at 30 and 60 DAS, respectively), dry weight of shoots(1.87 g), fresh and dry weight of roots (1.94 and 0.97 g, respectively) and vigour index II(175.65). With respect to survival of seedlings in seeds treated with GA_3 at 100 and 200 ppm and BA at 100 ppm recorded highest cent per cent field survival whereas, untreated seeds recorded lowest values for all the characters studied.

KEY WORDS : *Ruta graveolens*, GA_3, Benzyl adenine, Ethrel, Vigour index

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Garden rue or common rue or 'herb-of-grace', botanically called as *Ruta graveolens* Linn. (family Rutaceae) is one of the important medicinal plant and has used to cure various ailments like kapha, vata, strangury, fever, flatulence and epilepsy, fresh plant act as scorpion and insects repellent, leaves and seeds boiled in olive oil and the mixture is rubbed for rheumatism pains and swellings in the tradition system of medicines since ancient times (Vaidyaratnam,1996).

Ruta is a genus of strong scented, erect, glabrous herbs or under-shrubs of 30-90 cm height distributed throughout the Mediterranean region and Temperate Asia. Garden rue, is a native of Balkan Peninsula and South Eastern Europe, which is sturdy perennial plant with much branched rounded stem. Leaves alternate, shortly petioled, ultimate segments oblong to spathulate, leaflet with numerous translucent small short stalked oil glands containing a very irritating volatile oil, have a strong, disagreeable odour and bitter, acrid, pungent taste; flowers small, 12 mm in diameter, yellowish in corymbs, strongly aromatic; petals 4-5, spoon shaped with dentate or wavy margin; capsules small with lobes somewhat rounded (Prajapathi *et al*., 2003).

The plant contains pilocarpine, which is used to induce abortion in horses. The fresh herb on steam distillation yields a pale or greenish volatile essential oil (0.06%) called rue oil, the oil is rich in methyl ketone (80-90%) and is used for the preparation of methyl-n-nonyl acetaldehyde, widely used as a synthetic perfume and also many number of products like rue gel, rue tea, human growth complex, *Ruta graveolens*- Boiron single remedy, *Ruta graveolens* capsules have been produced by the drug industries.

Garden rue grows well at higher altitudes and can also be grow in medium elevations. It prefers a well drained calcareous clay soils commercially this plant is propagated through seeds, but there is a problem of seed germination and survivability of seedlings. Therefore, in the present study an effort has been made to improve the germination of seeds by different pre-sowing treatments. Synchronization and rapid emergence of seedlings are the commonly reported benefits of pre-sowing treatments. Effect of growth regulators on germination and seedling growth has been known for quite some time and numerous investigations are still going on. Gibberllins, cytokinins and ethrel have been successfully used for breaking the dormancy of seeds in various crops (Hartman *et al*., 2002) and hence, these are being tried to improve the seed germination and survival of garden rue.

RESEARCH METHODS

The present investigation was carried out in Sugandhavana, Aromatic crop section, at Division of Horticulture, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bengaluru, during 2011-12. The Division of Horticulture is located at an altitude of 930 meters from mean sea level, at a latitude of 12^0 58 North and a longitude of 77^0 35 East. Mean maximum temperature during the period of experiment was ranged from 28.0^0C to 34.6^0C, while the mean minimum temperature ranged between 14.3^0C to 21.2^0C. Similarly, the maximum relative humidity of 73 per cent was recorded during July and lowest of 57 per cent was recorded in March. So also, the rainfall varied from 8.6mm (April) to 95.8 mm (July) 2012.

The experiment was carried out in Completely Randomized Design with fourteen treatments along with control. Twenty five seeds were used for each treatment, which was replicated thrice. The seeds were treated by soaking it with GA_3 (50, 100, 200, 300, 400 ppm), benzyl adenine (25, 50, 100 and 200 ppm), ethrel (100, 200, 300 and 400 ppm) and distilled water (Control) for 3 hours. After pre-sowing treatment, the seeds were shade dried for 10 minutes and were sown in seed pans containing media 2:1:1 ratio (sand, soil and FYM) at 0.5 cm depth 2 cm apart and were kept in the poly house. The seed pans were watered daily with the help of rose can. Observations were recorded daily on germination parameters and monthly for vegetative parameters like plant height, number of leaves, stem girth, fresh weight, dry weight by keeping the seedlings in hot air oven at the temperature of 60^0C till constant weight was attained and seedling vigour for up to 90 days after sowing. The survival percentage of seedlings was also studied by counting the established seedlings from of the seedlings transplanted to the field in each of the treatment and their survival percentage was worked out. The data collected from the five labelled seedlings in each treatment were averaged and completely randomised design (CRD) was employed to find out the significance among different treatments with the help of 'F' test (Sunderaraju *et al*., 1972). The rate of germination and seedling vigour was calculated based on the following formulas (Bewley and Black, 1982).

$$C = \frac{\Sigma G n}{\Sigma G_n . D_n} \times 100$$

where,

G_n – Number of seeds germinated on a day n

D_n – Days from initial sowing

Vigour index – I (cm) = Mean seedling length X per cent germination

Vigour index – II (g) = Dry weight of seedling X per cent germination

RESEARCH FINDINGS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarised under following heads:

Seed germination characters:

Early germination (Table 1) was noticed in the seeds treated with GA_3 at 300 ppm and 400 ppm (6.0 days). This may be due to instigative action of GA_3 for germination of seeds. GA_3 induces the de-nova synthesis of proteolytic enzymes like amylase and ribonuclease, amylases in turn hydrolyse starch in the endosperm, providing the essential sugars for the early initiation of growth process (Copeland and Mc Donald, 1995). The other pre-sowing treatments like benzyladenine and ethrel might have also affected and altered the enzymatic reactions involved in germination process. Thus, the enhanced enzymatic reactions along with suppression of inhibitors might have acted in the faster germination and rate of germination due to pre-soaking treatments. Similar results were reported by Vasundhara *et al*. (2006) in roselle (*Hibiscus sabdariffa*).

Gibberellic acid (GA_3) at 400 ppm induced higher percentage of germination (89.33%) compared to other treatments. This might be due to the action of gibberellic acid which is known to overrule the photodormancy, thermo-dormancy, dormancy imposed by incomplete embryo development, machanical barriers and presence of germination inhibitors (Diaz and Martin, 1971). Gibberellins help to increase the availability of stored material for growing embryo by increasing the amylase enzyme activities in the seeds which leads to the faster and more germination of seeds and the variation in germination in pre-soaking of seeds may be due to the stimulative effect of these chemicals on the seeds as well as enhanced the enzymatic process and suppression of inhibition along with synthesis of RNA, which resulted in higher germination. The results of the present investigation are similar to the findings of Lalith Kumar (2008) in tulsi, ashwagandha, periwinkle and kalmegh.

Rate of germination was found maximum in GA_3 at 300 ppm (10.27) and minimum was in control (6.13). These differences in rate of germination may be due to variation in seed weight, presence of hard seed coat and concentration of chemicals used which in turn might have affected number of days taken for germination on which rate of germination depends. The enhancement in rate of germination due to pre-treatment of GA_3 directly activates the embryo by stimulating the synthesis of protiens, m-RNA and the hydrolytic activities in embryo (Bewley and Black, 1982)

Vegetative characters:

The data (Table 2) showed significant increase in the shoot length at 30 and 60 DAS in GA_3 treatment at 400 ppm (12.30 cm and 16.33 cm, respectively) and lowest shoot length was recorded in control (5.67 cm and 10.67 cm, respectively). The maximum number of compound leaves at 30 and 60 DAS were recorded in seeds treated with GA_3 at 300 ppm (6.60 and

Table 1 : Effect of pre-sowing seed treatments on germination attributes in garden rue (*Ruta graveolens* Linn.)

Treatments	Days taken for commencement of germination	Days taken for 50 per cent germination	Germination percentage	Rate of germination
T_1-Control (Distilled water soaking)	12.33	16.67	36.00	6.13
T_2-Soaking in GA_3 at 50 ppm	9.67	11.67	61.33	8.45
T_3-Soaking in GA_3 at 100 ppm	7.67	10.00	66.67	9.13
T_4-Soaking in GA_3 at 200 ppm	6.67	11.00	74.67	9.29
T_5-Soaking in GA_3 at 300 ppm	6.00	9.67	81.33	10.27
T_6-Soaking in GA_3 at 400 ppm	6.00	9.00	89.33	10.26
T_7-Soaking in BA at 25 ppm	11.33	14.33	52.00	6.58
T_8-Soaking in BA at 50 ppm	11.33	14.67	54.67	7.21
T_9-Soaking in BA at 100 ppm	10.00	14.33	62.67	7.39
T_{10}-Soaking in BA at 200 ppm	9.67	13.67	58.67	7.42
T_{11}-Soaking in ethrel at 100 ppm	8.33	11.67	48.00	8.77
T_{12}-Soaking in ethrel at 200 ppm	8.33	11.67	52.00	8.59
T_{13}-Soaking in ethrel at 300 ppm	7.67	10.00	64.00	8.78
T_{14}-Soaking in ethrel at 400 ppm	9.67	12.67	57.33	7.58
S.E.$\pm$	0.50	0.62	3.15	0.28
C.D. (P=0.05)	1.46	1.81	9.12	0.81
C V (%)	9.80	8.84	8.89	5.88

Table 2 : Effect of pre-sowing seed treatments on seedling growth parameters and survival of seedlings in garden rue (*Ruta graveolens* Linn.)

Treatments	Shoot length (cm)		No. of compound leaves		Collar diameter (mm)		Root length (cm)	No. of roots	Survival of seedlings (%)
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	60 DAS	60 DAS	30 DAP
T_1-Control (Distilled water soaking)	5.67	10.67	4.07	7.13	2.20	2.60	6.77	11.07	83.33
T_2-Soaking in GA_3 at 50 ppm	7.50	13.47	4.40	7.33	2.60	3.33	8.30	16.00	88.89
T_3-Soaking in GA_3 at 100 ppm	8.43	15.43	5.40	9.33	2.87	3.53	9.45	17.67	100.00
T_4-Soaking in GA_3 at 200 ppm	8.80	14.67	5.80	9.33	2.20	3.80	9.36	17.33	100.00
T_5-Soaking in GA_3 at 300 ppm	10.60	15.53	6.60	10.27	3.07	3.80	9.50	22.40	94.44
T_6-Soaking in GA_3 at 400 ppm	12.30	16.33	6.20	10.20	3.60	4.53	9.50	25.67	94.44
T_7-Soaking in BA at 25 ppm	7.53	13.33	5.00	8.13	2.20	3.67	7.23	22.33	88.89
T_8-Soaking in BA at 50 ppm	7.83	14.70	5.33	8.53	2.33	3.80	8.00	22.53	94.44
T_9-Soaking in BA at 100 ppm	8.00	15.87	5.49	8.20	2.40	3.27	8.73	23.67	100.00
T_{10}-Soaking in BA at 200 ppm	8.63	15.23	5.00	8.47	2.40	3.33	8.57	23.33	83.33
T_{11}-Soaking in ethrel at100 ppm	7.47	12.67	4.87	7.07	2.47	3.07	8.30	19.67	83.33
T_{12}-Soaking in ethrel at 200 ppm	7.07	10.97	5.27	8.07	2.53	3.67	7.23	19.47	94.44
T_{13}-Soaking in ethrel at 300 ppm	6.73	11.43	5.33	7.53	2.60	3.67	6.63	19.33	83.33
T_{14}-Soaking in ethrel at 400 ppm	6.37	10.90	4.13	7.27	2.47	3.40	6.20	17.60	83.33
S.E.+	0.65	0.86	0.34	0.47	0.17	0.21	0.48	2.15	3.93
C.D. (P=0.05)	1.88	2.49	1.00	1.35	0.48	0.60	1.39	6.24	11.38
C V (%)	13.91	10.91	11.46	9.67	11.21	10.15	10.21	18.78	7.42

DAS- Days after sowing, DAP- Days after planting

10.27, respectively) and also minimum number of compound leaves were recorded in control (4.07 and 7.13, respectively). Significant increase in shoot length and number of leaves might be due to early germination and a better seedling vigour caused by GA_3 treatments. The regulation of growth by gibberellins and benzyl adenine relates almost extensively to its stem elongation properties. Influence of gibberllic acid and benzyl adenine on stem elongation is by two ways; they have direct effect on stem elongation by inducing cell wall loosening, by increasing cell wall extensibility, stimulating the wall synthesis, reducing the rigidity of cell wall and by increasing cell division leading to more growth and the indirect effect of these chemicals on stem elongation is by increasing the synthesis of IAA (Leopold and Kriedemann, 1983). It is due to development of root system in seedling of these treatments in terms of its length and number, providing an efficient uptake of water and hence, a better seedling growth was recorded. Several workers have reported the action of GA_3 in inducing better shoot length crops like Kalmegh (Saraswathy *et al*., 2004). The production of more number of compound leaves in seedlings may be due to the vigorous growth induced by the GA_3 and benzyl adenine treatments, more number of branches which in turn facilitates better harvest of sunshine by the plants to produce more number of compound leaves.

The maximum collar diameter was recorded at 30 and 60 DAS in GA_3 (400 ppm) treated seeds (3.6 mm and 4.53 mm, respectively) and minimum was in the control (2.20mm and 2.60 mm, respectively). Gibberellins are found to influence the root growth, both in terms of increasing root length and their number per plant. Application of GA_3 at 400 and 300 ppm resulted in maximum root length (9.50 cm) while, GA_3 at 400 ppm was responsible for the maximum number of roots (25.33) per plant and the minimum root length and root number were recorded in control (6.20cm and11.07, respectively). The present findings are similar to that of Parameshwari and Srimathi (2008) in aonla. Significant increase in the survival of seedling was observed in seeds treated with GA_3 at 100, 200 and BA at 100 ppm (100%) and lowest was recorded in T_1, $T_{10,}$ T_{11} T_{13} and T_{14}, (83.33%). However, there was no significant difference among the concentrations of GA_3 treated seeds. Results obtained with respect survival of seedlings were matched with the findings Vasundhara *et al.* (2006) in roselle.

Fresh and dry weight of shoot was found maximum in GA_3 at 400 and 300 ppm (5.8 g and 1.87 g, respectively) (Table 3). While, the minimum was in control (2.43 g and 0.62 g, respectively). All the pre-sowing treatments increased the seedling fresh and dry weights as compared to control. The increased weight of seedling was mainly attributed to enhanced germination, early seedling emergence and better seedling growth. Application of GA_3 resulted in maximum fresh and dry weights of shoots which may be attributed to robust growth of seedlings. The robust growth of seedling might have also caused increased cell number and cell weight due to elongation and division of cell by gibberellins, which is in conformity with the results obtained by Velmurugan *et al.* (2003) in ashwagandha. With respect to fresh and dry weight of roots GA_3 at 300 ppm recorded the highest values (1.94g and 0.97g, respectively), than the any other treatments. However, it was at par with T_9 (BA 100 ppm) and the lowest was recorded in control (0.98 g and 0.33g, respectively). Increase in the fresh and dry weight of roots may also be due

Table 3 : Effect of pre-sowing seed treatments on seedling biomass and vigour at 60 days after sowing in garden rue (*Ruta graveolens* Linn)

Treatments	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of roots (g)	Dry weight of roots (g)	Shoot to root ratio	Vigour index (1)	Vigour index (2)
T_1-Control (Distilled water soaking)	2.43	0.62	0.98	0.33	1.61	632.67	33.95
T_2-Soaking in GA_3 at 50ppm	2.83	0.70	1.09	0.50	1.64	1330.13	73.57
T_3-Soaking in GA_3 at 100ppm	4.22	1.05	1.77	0.43	1.62	1232.80	98.91
T_4-Soaking in GA_3 at 200ppm	4.60	1.38	1.82	0.56	1.55	1789.47	143.15
T_5-Soaking in GA_3 at 300ppm	5.38	1.87	1.94	0.97	1.63	2027.33	175.65
T_6-Soaking in GA_3 at 400 ppm	5.81	1.77	1.79	0.80	1.72	2299.47	158.46
T_7-Soaking in BA at 25 ppm	4.90	1.60	1.13	0.42	1.84	1070.80	104.64
T_8-Soaking in BA at 50 ppm	5.08	1.87	1.70	0.63	1.83	1240.40	136.35
T_9-Soaking in BA at 100 ppm	4.65	1.80	1.81	0.93	1.81	1540.67	170.89
T_{10}-Soaking in BA at 200 ppm	4.56	1.63	1.56	0.61	1.77	1396.00	131.31
T_{11}-Soaking in ethrel at 100 ppm	3.56	1.80	1.35	0.66	1.52	1007.33	117.07
T_{12}-Soaking in ethrel at 200 ppm	3.64	1.60	1.14	0.43	1.51	945.80	101.92
T_{13}-Soaking in ethrel at 300 ppm	3.55	1.53	1.03	0.47	1.72	1155.20	129.23
T_{14}-Soaking in ethrel at 400 ppm	3.04	1.17	1.05	0.45	1.81	969.93	92.36
S.E.±	0.43	0.14	0.09	0.03	0.07	174.69	16.84
C.D. (P=0.05)	1.23	0.42	0.26	0.07	0.21	506.07	48.80
C V (%)	17.74	17.07	11.22	7.42	7.55	22.73	24.50

to robust root growth from GA_3 and benzyl adenine treaments. These results are supported by the findings of Wikum *et al.* (2009) in *Datura* species.

The highest vigour index (1) was found in GA_3 400 ppm (2299.47) and vigour index (2) was recorded highest in the GA_3 at 300 ppm (175.65) and lowest was in control. With respect to shoot to root ratio was recorded highest in the GA_3 treated seed, this may be due to the increased in the shoot and root length in the GA_3 treated seeds. The highest shoot to root ratio (1.84) was recorded in the BA at 25 ppm which was at par with GA_3 at 50, 300 and 400 ppm and BA at 50, 100, 200 ppm. While, control recorded lowest (1.61).While GA_3 100, 200 and BA at 100 ppm was responsible for the highest survivability of seedlings. This may be due to the vigorous growth of seddlings helps to establish well in the field.

Conclusion:

From the present investigation, it was concluded that application of growth regulators like GA_3, BA and ethrel increased the seed germination, seedling growth and survival of seedlings than the control. Among these, GA_3 at 400 ppm gave best results by recording early germination and maximum percentage of germination, while GA_3 100, 200 and BA at 100 ppm was responsible for the highest survivability of seedlings.

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