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Influence of Different Seed Treatments on Seed Quality Enhancement in Stevia (*Stevia rebaudiana* Bertoni.)

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A B S T R A C T

Stevia is perennial herb belongs to asteraceae family. It is popularly known as sweet leaf, candy leaf, sweet herb and honey leaf which are used to treat diabetes to bring down glucose levels, enhance insulin secretion and in various pharmaceutical formulations. A laboratory experiment was conducted at University of Horticultural Sciences, Bagalkot during 2019-2020 to assess the seed quality parameters of stevia by treating the seeds with different treatments. The experiment was laid out in a Completely Randomized Design (CRD) with ten treatments in four replications. The seeds of stevia treated with GA_3 at 10 ppm soaked for 12 hours had took minimum days for first seed germination (3.00 days), recorded maximum germination (62.25%), root length (10.30 mm), shoot length (14.15 mm), seedling vigour index (1522), seedling dry weight (2.90 mg per 10 seedlings) and field emergence (47.75%). The growth regulator GA_3 at 10 ppm found to be superior in improvement of seed quality parameters of stevia compared to other treatments.

Introduction

Stevia (*Stevia rebaudiana* Bertoni.) is a natural calorie free bio sweetener; it is indigenous to the Amambay region in the Northeast of Paraguay in South America (Alahmad, 2018). The crop stevia is widely dispersed in the neighbouring parts of Brazilian Highlands, Argentina, Central America, South American Andes and South-East Asia. An American member of the genus stevia has chromosome number of 2n = 22 and it needs a specific photoperiod of 12 to 16 hours for vegetative growth (Frederico *et al*., 1996). In India, it can be grown very excellent in states like Maharashtra, Punjab, Chhattisgarh, Karnataka, Madhya Pradesh and Andhra Pradesh. The climatic conditions of these states are very much suitable to get good yield and quality. *Stevia rebaudiana*, as a non-nutritious natural sweetener has risen as a safe sugar substitute for human health. In addition to the sweetening potential, it has numerous therapeutic advantages, as it has diuretic (Bhutia and Sharangi, 2016), anti-hypertensive (Chan *et al*., 1998), anti-hyperglycemic (Jeppesen *et al*., 2003), anti-diarrheal and immune modulator properties (Chatsudthipong and Muanprasat, 2009). The sweet components of stevia are called steviol glycosides which are found in the stevia leaf, used in an extensive range of food items as a non-calorie sweetener. There are different classes of steviol glycosides of which rebaudioside (13%) and stevioside (3-10%) are most abundant substances (Tavarini and Angelini, 2013).

Stevia demand in herbal world and as raw drugs is increasing day by day, which leads for large scale cultivation and which warrants for huge demand of seeds and planting materials. Stevia is propagated through vegetative cuttings and supply required quantity of planting materials is quite difficult as requires longer period for propagation. Whereas, propagation by seeds is problematic, because of low germination, due to very small endosperm, infertile in nature and produce variable percentage of viable seed. The information related to germination and seed quality testing of stevia is very meager. Seeds treatment with growth regulators and other substances may regulate the physiological and biochemical process that may initiates seed germination. Hence, the present study was conducted to study the influence of different seed treatments on seed quality enhancement in stevia (*Stevia rebaudiana* Bertoni.).

Materials and Methods

The experiment was conducted to study the influence of different seed treatments on seed quality enhancement in stevia (*Stevia rebaudiana* Bertoni.) at Seed Unit, University of Horticultural Sciences, Bagalkot during the year 2019-2020. The experiment was laid out in Completely Randomized Design (CRD) with ten treatments in four replications. Freshly harvested seeds of stevia were collected from the farmer's field and black colour, fertile seeds were used for the study.

In the present study, the treatment used are T_1: Control (Untreated), T_2: Water soaking for 24 hours, T_3: IAA @ 5 ppm for 12 hours, T_4: IAA @ 10 ppm for 12 hours, T_5: GA_3 @ 5 ppm for 12 hours, T_6: GA_3 @ 10 ppm for 12 hours, T_7: Kinetin @ 5 ppm for 12 hours, T_8: Kinetin @ 10 ppm for 12 hours, T_9: Humic acid @ 5% for 12 hours, T_{10}: Cow urine @ 40% for 24 hours. After completion of soaking period, the treated seeds were separated from solution and dried under partial shade and brought back to original moisture content. The seed germination test was performed using petri plate method (top paper method) as described by ISTA (International Seed Testing Association) rules. The treated seeds were kept on the petri

plates lined with Whatman No. 1 filter paper moistened with 5 ml of distilled water to ensure sufficient moisture for seed germination. Further they were kept in seed germinator at 25±2º C temperature and 85±3 per cent relative humidity. Observations were recorded on 15th day for different seed quality parameters. For statistical analysis software used was WASP (Web Agricultural Statistics Software Package).

Results and Discussion

The stevia seeds treated with seed treatments were showed significant difference with respect to seed quality parameters. The results obtained for different seed quality parameters are discussed here under.

The seeds treated with GA_3 at 10 ppm and soaked for 12 hr took minimum number of days for first seed germination (3.00 days), whereas, treatments like GA_3 at 5 ppm soaked for 12 hr (3.00 days), kinetin at 5 ppm soaked for 12 hr (3.00 days), kinetin at 10 ppm soaked for 12 hr (3.00 days), IAA at 5 ppm soaked for 12 hr (3.00 days) and water soaked for 24 hr (3.25 days) also took lesser number of days for seed germination. Maximum number of days to first seed germination was recorded in the treatment with cow urine 40 per cent soaked for 24 hr (5.00 days) and IAA at 10 ppm soaked for 12 hr (5.00 days).

Exposure of GA_3 recorded early days to commencement of germination, this might be due to it's instigate action on embryo and tends to cause de nova synthesis of hydrolyzing enzymes mainly amylase and protease. Thus, hydrolyzed food is used for embryo development which results in subsequently initiation of early seed germination due to the flow of endogenous auxin and gibberellins like substances (Gurung *et al*., 2014). These results are in accordance with Chauhan and Nautiyal (2007) in jatamansi, Khanna *et al*. (2013) in ashwagandha and Simlat *et al*. (2019) in stevia.

Higher seed germination per cent was recorded in seeds treated with GA_3 at 10 ppm soaked for 12 hr (62.25%) and followed by the treatment GA_3 at 5 ppm soaked for 12 hr (57.50%). However, the cow urine at 40 per cent soaked for 24 hr showed least seed germination per cent (15.75%). Growth regulator GA_3 shown maximum germination per cent, this may be due to involvement of GA_3 in enzyme synthesis, one of them is amylase which participates in the breakdown of starch, lipids and protein which transforms the insoluble starch into soluble sugars in endosperm during the course of germination. Thus, these sugars provide energy to seeds that is needed for germination related physiological and metabolic process. GA_3 also boosts elongation of cells, such that radical can push through endosperm of seeds which enhances in germination (Barathkumar, 2019). Similar reports with respect to seed germination are recorded by Enkeshwer *et al*. (2010) in stevia, Simlat *et al*. (2019) in stevia and Sapra *et al.* (2020) in ashwagandha.

Among different seed treatments, root length was found to be higher in the seeds treated with GA_3 at 10 ppm soaked for 12 hr (10.30 mm), which was on par with kinetin at 10 ppm soaked for 12 hr (10.10 mm) and followed by GA_3 at 5 ppm soaked for 12 hr (9.50 mm). Minimum root length was noticed in humic acid at 5 per cent soaked for 12 hr (7.85 mm). During early stage of the seed germination, the exogenous application of growth regulator GA_3 induced gluconeogenic enzymes activities and improvement in germination characteristics thus reflected in terms of increase in highest root length (Jain *et al*., 2017). Contrary reports were observed by Ambika *et al*. (2019) in henna.

Significantly, maximum shoot length was recorded in GA_3 at 10 ppm soaked for 12 hr (14.15 mm) and followed by kinetin at 10 ppm soaked for 12 hr (13.10 mm). While, the lowest shoot length (9.35 mm) was noted in humic acid at 5 per cent soaked for 12 hr. The highest values of shoot length obtained with GA_3 might be due to the improved osmotic uptake of the nutrients cause cells to multiply and elongation in the cambium tissue, which initiates the metabolic activities, helps for growth of shoot length and nullifies the growth inhibitors in seeds (Singh *et al*., 1989). Similar reports were observed by Ambika *et al*. (2019) in henna.

Seedling vigour index was considerably higher in the treatment GA_3 at 10 ppm soaked for 12 hr (1522) and followed by GA_3 at 5 ppm soaked for 12 hr (1268), while, IAA at 10 ppm soaked for 12 hr showed least seedling vigour index (286).The seedling vigour index was obtained maximum in seeds subjected with GA_3, due to higher metabolic activity, respiration rates, better utilization and movement of metabolites to growth point. Metabolic process such as sugar mobilization, oxidation and protein hydrolysis are induced by enzymatic and hormonal mechanism, leads to germination, elongation in length of root and shoot which in turn increases the seedling vigour index (Patel *et al*., 2018). Similar results were also confirmed by Ambika *et al*. (2019) in henna and Sapra *et al.* (2020) in ashwagandha.

Maximum seedling dry weight was noticed in GA_3 at 10 ppm soaked for 12 hr (2.90 mg per 10 seedlings), which was on par with kinetin at 10 ppm soaked for 12 hr (2.75 mg per 10 seedlings) and followed by the treatment GA_3 at 5 ppm soaked for 12 hr (2.58 mg per 10 seedlings). Cow urine at 40 per cent soaked for 24 hr recorded lowest seedling dry weight (1.03 mg per 10 seedlings).

The exogenous soaking of stevia seeds in GA_3 for 12 hours produced maximum dry weight of the seedlings, this might have association with overall growth of seedling. Increased growth of seedlings has resulted into the absorption and redistribution of food material in the seedling. The higher mobilization and accumulation of nutrients has lead to elongation of cell, root and shoot length helps for more fresh weight of seedlings. Thus growth of seedlings is results in increase of seedling dry weight (Thorat *et al*., 2017). These results are in acceptance with findings of Ambika *et al*. (2019) in henna and Sapra *et al*. (2020) in ashwagandha.

Seeds treated with GA_3 at 10 ppm soaked for 12 hr showed maximum field emergence per cent (47.75%), which was on par with GA_3 at 5 ppm soaked for 12 hr (45.25%) and followed by the treatment kinetin at 5 ppm soaked for 12 hr (41.50%). Whereas, the treatment cow urine at 40 per cent soaked for 24 hr (10.50%) recorded minimum field emergence per cent. Field emergence was exhibited highest in GA_3, one of the main reason is due to maintenance of hormonal balance and suppressing inhibitors of seeds. GA_3 is important for stimulation of embryo growth in turn aids for germination of seeds and also helps for production and secretion of hydrolytic enzymes from aleurone cells. Thus, enzymes moves to endosperm storage reserves that boosts for emergence of seed germination and growth (Shariatmadari *et al*., 2017).

In conclusion, present study indicates that GA_3 play roles in controlling seed germination and seedling growth of Stevia. Stevia seeds treated with GA_3 at a concentration of 10 ppm found to be superior in improvement of seed quality parameters (Table 1).

Table.1 Seed quality parameters as influenced by seed treatments in stevia

Treatments	Days to first seed germination	Seed germination (%)	Root length (mm)	Shoot length (mm)	Seedling vigour index	Seedling dry weight (mg per 10 seedlings)	Field emergence (%)
T_1- Control (untreated)	4.00	39.25 (38.79)	8.00	9.85	700	1.33	29.50 (32.89)
T_2- Water soaking for 24 hr	3.25	44.25 (41.70)	8.50	11.15	869	1.83	35.25 (36.42)
T_3- IAA @ 5 ppm for 12hr	3.00	47.75 (43.71)	8.55	11.65	965	2.00	38.25 (38.20)
T_4- IAA @ 10 ppm for 12 hr	5.00	16.25 (23.75)	7.90	9.70	286	1.13	11.75 (20.02)
T_5- GA_3 @ 5 ppm for 12 hr	3.00	57.50 (49.32)	9.50	12.55	1268	2.58	45.25 (42.27)
T_6- GA_3 @ 10 ppm for 12 hr	3.00	62.25 (52.09)	10.30	14.15	1522	2.90	47.75 (43.71)
T_7- Kinetin @ 5 ppm for 12 hr	3.00	54.25 (47.44)	9.00	12.30	1156	2.20	41.50 (40.11)
T_8- Kinetin @ 10 ppm for 12 hr	3.00	51.75 (46.00)	10.10	13.10	1201	2.75	40.00 (39.23)
T_9- Humic acid @ 5% for 12 hr	4.00	40.25 (39.38)	7.85	9.35	692	1.50	24.50 (29.66)
T_{10}- Cow urine @ 40% for 24 hr	5.00	15.75 (23.34)	8.35	10.5	297	1.03	10.50 (18.88)
S. Em±	0.08	0.86 (0.55)	0.13	0.17	21.00	0.05	0.74 (0.49)
C.D. at 1%	0.31	3.36 (1.60)	0.49	0.65	80.00	0.18	2.88 (1.91)

(Values in the parenthesis are arc sine root transformed values)

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