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Utilization of Micropropagation and Mutation Breeding in stevia Improvement so far-A Critical Review

Tabassum*, Jaspreet Kaur Basati, Parbhjot Kaur and Gurdeep Singh
Faculty of Agriculture, Baba Farid College, Baba Farid Group of Institutions, Bathinda (Punjab), India.

(Corresponding author: Tabassum)*
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ABSTRACT: Stevia is a perennial herb, widely used as a non-toxic and non-caloricnatural sweetener in many countries around the world as the stevioside extract from the leaves taste 300 times sweeter than cane sugar. The seeds are smaller in size and the germination percentage is very low with a significant problem of low fertility which is primarily a constraint of self-incompatibility. Propagation by seeds does not allow the production of homogeneous populations and generate variability among individuals of a population in important features like sweetening levels and composition. Propagation by seeds does not allow the production of homogeneous populations which generate variability among individuals of a population in important features like sweetening levels and composition. Vegetative propagation is also limiting by the fact that a limited number of plants can be generated from a single plant, therefore is a limiting factor for rapid multiplication. Due to these difficulties, tissue culture is an important alternative for rapid multiplication of stevia plants for enhanced production and along with mutation breeding to develop a new variety with improved characters. Modern techniques such as molecular markers, HPLC can also be explored to speed up breeding programme for higher yield and glycoside content. This review article enlightens use of mutation breeding and tissue culture for stevia improvement so far in order to focus on it as an effective breeding strategy in case of this crop.

Keywords: Stevia, Mutation Breeding, Micro-propagation, Natural non-caloric sweetener.

INTRODUCTION

The Stevia plant (*Stevia rebaudiana* Bertoni), belongs to asteraceae family (Aster/Sunflower family), a perennial herb native to north eastern Paraguay (Xu *et al.,* 2021) which is cultivated for its high economic value due to sweetness. It is a natural sweetener plant popularly known as sweet weed, sweet leaf of Paraguay, sweet herbs and honey leaf, candy leaf, and honey yerba, which is estimated to be 300 times sweeter than cane sugar (Yadav *et al*., 2011). *S. rebaudiana* is one of the 154 members of genus *Stevia* which produces sweet steviol glycosides (Robinson, 1930; Brandle *et al.,* 1998). The leaves can be used fresh, dried to sweeten beverages or desserts and can be commercially processed into powdered zero-caloric sweeteners (Modi *et al*., 2011; Yesmin, 2019). Stevia is a diploid plant, having 11 pairs of chromosomes (2n=22), self-incompatible with entomophilous pollination (Yadav *et al*., 2011; Xu *et al.,* 2021). Plant grows up to 65-80 cm height with sessile, oppositely arranged leaves, white small flowers seen in terminal inflorescence (Yesmin, 2019). The flower contains 5 to 6 seeds with two different colours, black (viable) and tan (non-viable). The seeds are contained in 3 mm length of achenes, which has 20 persistent pappus bristles (Goettemoeller and Ching 1999).

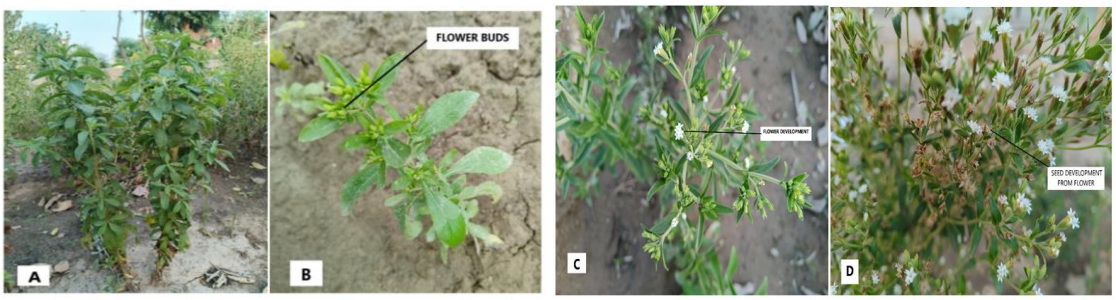

Fig. 1. Morphology of *Stevia rebaudiana* plant at different growth stages, (A) Vegetative growth with leaves and branches (B) Flower buds development (C) White coloured flowers bearing branches (D) Seed development.

Out of all the cultivated species, *S. rebaudiana* is the sweetest of all. It grows best in upland areas in sub-tropical climate but in other places it can be grown as an annual (Kumar and Mishra 2015). Some other important related species of *Stevia rebaudiana* includes *Stevia eupatoria*, *Stevia lemmonii* (Lemmon's stevia), *Stevia micrantha* (Annual stevia), *Stevia ovata* var. *texana* (Roundleaf candy leaf), *Stevia plummerae* (Plummer's stevia), *Stevia plummerae* var. *alba*, *Stevia rhombifolia* (Kunth), *Stevia salicifolia* (Willow-leafstevia), *Stevia serrata* (sawtooth stevia), *Stevia viscida* (viscid stevia), *Stevia commixta*, *Stevia satureiaefilia*, *Stevia leptophylla*, *Stevia myriadenia*, *Stevia ophryphylla*, *Stevia selloi*, *Stevia nepetifolia*, *Stevia oligophylla*, *Stevia origanoides* and *Stevia triflora* (Yadav *et al.,* 2011).

Stevia was known to the Spanish in the 16th century. However, it remained insignificant until in 1888, it was rediscovered by Dr. M. S. Bertoni in Paraguay. Then in 1905, the plant was scientifically described and named in honour of a Paraguayan chemist, Dr. Rebaudi. It is significantly cultivated in countries such as China, Brazil, Columbia, Paraguay, Indonesia, India, Japan, Korea, USA, Tanzania, Canada (Yadav *et al.,* 2011; Cosson *et al.,* 2019) and recognized as a healthier alternative to sugar in the world. In India, it is largely cultivated in several areas of Rajasthan, Karnataka, Oddisa, Maharashtra and Kerala (Kumar and Mishra 2015).

Stevia leaves have been used for more than 1,500 years by the Guarani people to sweeten the tea traditionally, it also had a number of applications in folk medicine. The first scientific record of the plant dates back to 1887, when Dr Moies Santiago Bertoni described the biological properties of Stevia (Hossain *et al.,* 2017). In 1971, Japanese scientists developed the first commercial stevia-derived sweetener, which quickly gained popularity in that country (Crammer and Ikan, 1986). From past few years, the up regulated demand of natural sweetners has motivated the farmers towards cultivating stevia at large scale.

A systemic classification of *Stevia rebaudiana* is given below Yadav *et al.* (2011):

Kingdom-Plantae
Division-Magnoliophyta
Class-Magnoliopsida
Group-Monochlamydae
Order-Asterales
Family-Asteraceae
Tribe-Eupatorieae
Genus-*Stevia*
Species-*rebaudiana*

The main active compounds of the *Stevia* are the *Stevioside* and *Rebaudioside* which are reported to be 150 times sweeter than sugar (Cardello *et al*., 1999; Ahmad *et al.,* 2019). Presently, consumption of sugar alternatives such as low-calorie stevia sweetener is on the rise due to increasing awareness about the effects of sugar intake, as these are believed to help in improving population heath by inducing weight loss and weight management. Steviol glycosides are non-glycemic (i.e., they do not affect blood glucose levels), not fermentable, heat/pH stable and the human body does not metabolize these glycosides , so it contains zero calories as a non-nutritive sweetener (Geuns *et al.,* 2006; Samuel *et al.,* 2018). On the other hand, artificial sweeteners are mostly developed from synthetic compounds in the laboratory and most of them have safety issues, aspartame is an example of a widely used artificial sweetener in the food and beverage industries, however, it is not heat stable and its ingestion may have serious negative side effects associated with carcinogenicity and brain disorders and its components can lead to a number of other health problems (Tandel, 2011). Thus, it is advisable to prefer natural sweeteners over them. Stevia is being utilized as a complementary to sugar and it possesses high commercial potential due to excellent taste profile, synergistic with sugar and other high intensity sweeteners, non-calorie, suitable for consumption by diabetic patients with no major safety concerns. High purity stevia extracts are approved for use as a sweetener worldwide.

Stevia rebaudiana shows immense potential as an agricultural crop for the development of a high potency sweetener. The steviol glycosides, particularly rebaudioside A, extracted from the leaves of stevia received great attention currently due to its most desirable sweetness and safety profile. Rebaudioside-A has the least bitterness of all the steviol glycosides in the *S. rebaudiana* plant. Therefore, development of new varieties of *S. rebaudiana* with a higher content of rebaudioside-A and a reduced content of stevioside is the primary aim of plant breeders concerned with the improvement and utilization of this source of natural sweeteners. Conventional plant breeding approaches such as selection and inter-crossing among various desirable genotypes is the best method for improving quality traits in this highly cross-pollinated crop. Various plant types with larger amounts of specific glycoside have already been patented, such as RSIT 94-1306, RSIT 94-75, RSIT 95-166-1 through selection and inter-crossing. Synthetic and composite varieties like AC Black Bird and PTA-444 have been developed (Yadav *et al.,* 2011). Al-Taweel *et al*. (2021) reviewed the data of previous crop improvement studies by traditional breeding and biotechnological approaches. *S. rebaudiana* plants are conventionally propagated through cuttings, but this traditional method cannot produce a large number of plants. The seeds of this species are smaller in size and the germination percentage is very low with a notable problem of low fertility. One of the reasons for lower fertility indices is presence of self-incompatibility. Therefore, modern techniques of propagation such as *in vitro* regeneration by tissue culture are needed to enhance the production for this important species. Mutation induction could play a significant role in the stevia improvement programs and polyploids could also be of great importance as they showed larger leaves and potential for higher SVgly than the standard diploid (Al-Taweel, *et al*., 2021). Therefore, keeping these points in view, this review is abstracting and concluding the available research data as scientific literature on the utilization of micro propagation and mutation breeding for mass multiplication of stevia and further crop improvement and increase SVgly content.

Micropropagation technology for stevia improvement. Poor germination percent and presence of self-incompatibility are major limiting factors for large scale stevia cultivation. Although propagation is generally done by stem cuttings, which rooting easily but requires high labour inputs and that makes it costly (Goettemoeller and Ching 1999). Despite poor germination, seed propagation results into heterogenous plant population with genetic as well as phenotypic variability for many economically important traits like SVgly and stevio sides content. In this regard, from past few years, micropropagation is playing important role for rapid mass multiplication of stevia. Singh *et al.* (2017) compiled micro propagation methods and protocols used by previous researchers and concluded that conventional propagation methods are not produce adequate planting material in stevia due to certain limitations. They reported plant tissue culture as the only technology to produce quality planting material in less time.

Many workers have utilized micropropagation in stevia. Ahmed *et al.* (2007) did clonal propagation in stevia in Bangladesh to generate shoots from nodal explant and regenerated multiple shootlets via axillary shoot proliferation in MS medium supplemented with 1.5 mg/L BA + 0.5 mg/L Kn. Alhady (2011) carried out the study for standardization of an effective in vitro protocol for rapid multiplication in Egypt by taking nodal stem cuttings as explant. They use MS medium supplemented with different concentrations of 6-benzylaminopurine (BA) individually or in combination with Kinetin (Kin) and recorded highest survival percentage (90%) as well as growth percentage to survival (100%) on MS medium supplemented with 0.5 mg/L BA + 0.5 mg/l (Kin). They concluded that type of cytokinine used was the most important factor affecting shoot multiplication as the highest shoot multiplication rate was obtained from single stem node segment cultured on medium supplemented with BA while, medium supplemented with kinetin resulted in elongated shoots. Sikdar *et al.* (2012) carried out experiment with an aim for establishing an efficient callus initiation and direct organogenesis system in stevia. They recorded that best callus regeneration was observed by taking nodes as explant. They used MS medium supplemented with α-naphthalene acetic acid (NAA) and 6-benzyladenine (BA) in various concentrations. Their results revealed that a concentration of 2.0 mg/L NAA + 2.0 mg/L BA showed the highest callus induction by nodal explants. They also concluded that leaf explants showed very poor callus. For direct organogenesis, MS medium supplemented with BA at 1.0 mg/L was best for multiple shoot proliferation. Razak *et al.* (2014) carried out micropropagation and developed a protocol for in vitro micropropagation using 6-benzylamino purine (BAP) and Kinetin (Kn) for the formation of multiple shoot proliferation and Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and 1-Naphthaleneacetic acid (NAA) for the induction of roots. They recorded maximum shoot formation on MS medium supplemented with 0.5 mg/L BAP and 0.25 mg/L Kn and maximum root formation on MS medium with 1.0 mg/L IBA. Luwanska *et al.* (2015) applied invitro propagation for obtaining stevia plantlets with higher glycoside content. They carried out comprehensive investigation in stevia regarding use of invitro micropropagation culture for the biosynthesis of secondary metabolites like stevioside and Reb A. Bhingradiya *et al.* (2016) studied effect of different media and plant hormones on the growth of stevia in vitro. They used several concentrations of BAP and NAA with both MS and WPM medium and concluded that WPM medium performed better then MS medium as it gave better growth in terms of number of shoots, shoots length, number of internodes, multiplication rate and callus induction. Pradhan and Dwivedi (2016) used half strength MS medium supplemented with different concentration of Kn, BAP (for shooting) and IAA, IBA (for rooting) to culture nodal segments for shoot proliferation. Almost in all the cultures shoot primordia initiation was observed 2-5 days after the inoculation. Half strength MS media+0.2 mg/L Kn produced maximum number of shoots with high shoot length and number of leaves, after 60 days of inoculation. Maximum number of roots with root length were recorded on media + 2.0 mg/L IBA+0.5 mg/L Kn. Micro-propagation using half-strength MS media can be more economical and can be effectively used for mass production of stevia under *in vitro* condition. Deshmukh *et al.* (2017) designed an efficient and economic medium for mass multiplication of Stevia. MS medium supplemented with BAP and Kinetin was used. They found that Auxiliary buds showed better sprouting than apical buds as former gave better response to the medium designed for spouting. BAP (2mg/L) + Kinetin (0.5mg/L) concentrations provided best results for shoot induction within a week and these shoots obtained 3-4 cm height with 2-3 weeks. and shoot grown upto height of about 3 to 4 cm in 2 to 3 weeks. Yesmin (2019) also used MS medium supplemented with BAP and Kn separately and in several combinations with NAA. They recorded maximum no of shoots on MS medium with 1.5 mg/l BAP + 5 mg/l NAA while, MS medium with 0.2mg/l IBA was found to be best for maximum root induction. *Invitro* regenerated plants grew normally without showing any morphological variation and flowered after 40 days of transplantation. Formation of callus is of paramount importance for both creating genetic variability as well as prompt mass multiplication. Masri *et al.* (2019) carried out study on stevia with an aim to establish an efficient method of callus formation and regeneration. They use three different explants *viz.*, shoot tip, leaf cuttings and nodal segment on MS medium with different concentrations and combinations of plant growth hormones. Results revealed that MS+1.0 mgl/L 2,4-D+0.75 mg/L NAA combination was best as it gave the highest fresh weight of callus and it regenerated a sufficient number of shoots also. For root formation, Half MS+1 mg/L IBA was found to be best as it gave 88.67% root formation with highest root length (2.90cm).

Mutation breeding. Mutation breeding is used to describe the deliberate induction of mutant lines for plant products development and crop improvement

(Chiew *et al.,* 2016) which provides an alternative way in getting desired characters that either do not exist naturally or have been missing during the evolutionary process (Novak and Brunner 1992). Mutation breeding practices are favoured over traditional breeding methods and genetically modified organisms as multiple traits mutants can be isolated unlike the transgenics where only a single trait can be introduced into the crop at a single attempt. In addition, mutation induction can help to establish mutant lines range and determine trait specific genes for the creation of a molecular gene database to support molecular functional genomics study and improve bioinformatics for future plant varieties development.

Induced mutation is aimed at increasing the mutation frequency rate for the sake of selecting suitable variants for plant breeding (Jain, 2010). Thus, mutation breeding provides an alternative way in getting desired traits or features that either do not exist naturally or have been missing during the evolutionary process (Novak and Brunner 1992; Chiew *et al.,* 2016).

Induction of mutation and mutagenic efficiency of mutagens. Mutation refers to the process in which heritable change in the genome or DNA of individuals occur as a result of treatment with mutagen. It has played a key role in evolution as the changes are heritable which give rise to the development of a new genotypes, species and genera (Mba *et al.,* 2010). The agents used to generate artificial mutation are known as mutagens and can be grouped into chemical and physical mutagens (Mba, 2013; Chiew *et al.,* 2016). If for a particular character, there is no genetic variability available naturally in the populations, genetic diversity can be created by mutation in both seed and vegetatively propagated crops by exposing botanical seeds and vegetative parts including stem cuttings, twigs buds, and tubers to mutagenic agents (Jain, 2010; Ulukapi and Nasircilar 2015). Both chemical and physical mutagens have been used to generate useful mutation but physical mutagens are more commonly used to develop mutant varieties. According to Forster *et al.* (2012) various factors *viz.,* source availability, accessibility, the suitability of the mutagens used, the safety of both treatment and post-treatment management and the cost of treatment can impact the performance of a particular mutagen. The two main cause for the more efficiency of physical mutagen in plant mutagenesis are energy and ability to penetrate the tissues. Both ultraviolet radiation (UV) and ionising radiations like X-rays, gamma-rays, neutrons, alpha and beta particles etc have been used so far but according to Jain, (2010) UV has moderate penetration capacity and induce fewer chemical changes, while, ionising radiations have better penetration capacity and can induce more chemical changes to plant tissues. Base analogues, intercalating agents, alkylating agents, nitrous acid, and chemicals that alter the structure of DNA are chemical mutagens and their effectiveness is mainly due to generation of point mutation, insertions, deletion, deamination, transitions, strand breaks resulted into the stoppage of transcription and replication (Jain, 2005; Toker *et al.,* 2007; Forster *et al.,* 2012). The most popular and widely used chemical mutagen is ethyl methane sulfonate which is a mono-functional alkylating agent, leads to high gene mutation with low chromosome aberration frequency (Chiew *et al.,* 2016).

Mutagenesis in stevia- A useful approach for genetic improvement. Mutation breeding changes structure of genes to accelerate the expression of desired plant traits more rapidly than conventional plant breeding approaches. Mutation have been generated in stevia by exposing them to various physical and chemical mutagens. The use of both chemical and physical mutagens allows a much faster way of obtaining genetic diversity within a plant population. Numerous mutagenic agents such as X-rays, gamma-rays, thermal neutrons, fast neutrons, chemicals like sodium azide (SA), ethyl methane sulfonate (EMS), diethyl sulphate (DES), methyl nitrosourea (MNU) have been reported to cause beneficial mutations in stevia (Al-Taweel *et al.,* 2021). One of the dominant advantages in induced mutations is that multiple traits mutants can be isolated unlike the transgenic way whereby only a solitary trait can be introduced into the crop. In addition, mutation induction can help to establish mutant lines range and determine trait specific genes for the creation of a molecular gene database to support molecular functional genomics study and improve bioinformatics for future plant varieties development (Chiew *et al.,* 2016). In various studies, colchicine has been found effective in developing new variants of stevia with two-fold increase in steviol glycosides contents both steviosides and Reb-A (Singh *et al.,* 2015).

In any mutation breeding programme, it is crucial to determine the effective dosage of mutagen applied to induce desirable genetic modifications with minimal undesirable effects to ensure the success of mutation induction (Kangarasu *et al.,* 2014). It has been observed that higher mutagen doses certainly bring mortality and sterility, while lower doses allow recovery of plants after treatment. Therefore, in order to obtain effective dosages of any mutagen to be used, determining lethal dose (LD50) is critical (Rajarajan *et al.,* 2016). LD50 is that dose of mutagen at which the highest frequency of mutation received with half of the treated population survived and half dead after treatment. The value of lethal dose of any mutagen may differ for one plant species to another, the LD50 of *Stevia rebaudiana* was reported to be 29 Gy (Kangarashu *et al.,* 2014).

Among all the physical mutagenic agents, the most frequently used mutagens in mutation breeding are gamma rays and x-rays (Mba, 2013). Gamma rays cause noticeable morphological alterations in plant tissues and other biochemical responses at cellular level (Hasbullah *et al.,* 2012; Hussein *et al.,* 2012). Gamma rays are the most energetic type of ionising electro-magnetic radiation, with short wavelength but high penetration capacity, interacting with atoms or molecules to create free radicals in the cells where these free radicals can destroy or alter plant cells components (Maamoun *et al.,* 2014). Forster *et al.* (2012) explained that the ionisation process can take place through three major mechanisms *viz.,* photoelectric effect, Compton scattering and pair production as gamma rays pass through the plant tissues. Depending on the irradiation

level, they influence germination of seed, morphology, anatomy, biochemistry and physiology of plants (Wi *et al.,* 2007). In recent past, development of useful mutants was being possible by application of gamma irradiation in plants, and it showed increasing potential in vegetatively propagated plants, especially in stevia (Predieri, 2001).

Many researchers used gamma irradiation for mutagenesis in stevia plants and recommended various doses based on their results. Snehal and Madhukar (2011) recorded low doses of gamma irradiation (5 kilorad (kR) to 15 kR) were more effective in variability generation in stevia whereas, high doses of gamma irradiation (25 kR and 30 kR) caused necrosis and delayed callus induction in explants. Likewise, Pande and Khetmalas (2011) also recorded that gamma rays (15 kR to 30 kR) significantly reduced seed germination and seedling survival rate in stevia. Exposure of stevia to gamma radiation has been proved useful for development of new varieties with improved SGs contents. Yang *et al.* (2013) referred that 60CO-γ and ion beam injections had mutagenic impacts on *S. rebaudiana* hybrid progenies as the treated plants showed lower height, consistent leaf shape, fewer branches, shorter internode length, lodging and cold resistance. Khalil *et al.* (2014) obtained *in vitro* shoots with higher stevioside content generated from gamma irradiated stevia seeds. On the basis of their findings, they proposed that the best organ for stevioside and Reb-A accumulation was the *in vitro* shoots as chromatographic data also revealed slight increment in the stevioside content. Noordin *et al.* (2014) studied effect of gamma irradiation on in vitro growth of stevia. They recorded highest shoot formation on MS media with 1 mg/L Kn. They also attempted to identify LD50 dose for in vitro mutagenesis in stevia and recorded 29 Gy for acute irradiation and 45 Gy for chronic irradiation. The effective doses selected were 10, 20, 30 and 40 Gy and were applied for in vitro mutagenesis of stevia shoots. Similarly, Nurhidayah *et al.* (2014) recommended 10 Gy, 20 Gy and 30 Gy as effective doses for *in vitro* mutagenesis of stevia shoots. Ali *et al*. (2014) used Cobalt-60 gamma ray to irradiate stevia seeds. It was observed that gamma radiation did not affect the germination of seeds but induced a higher suppresses root development. Khan *et al.* (2016) exposed stevia leaf explant to gamma rays and EMS to develop stevia mutants with higher stevioside and Reb-A content. Results revealed that gamma rays treated plants induced the double-fold Reb-A with lower stevioside content while plants exposed to EMS reported more than two-fold increase in both stevioside and Reb-A. They recorded higher expressions of enzymes from uridine diphosphate glucosyltransferase (UDP Glucosyltransferase) family involved in the steviol glycoside biosynthesis pathway in the mutant strains of stevia. UDP glycosyltransferases are a family of enzymes that catalyses the addition of the glycosyl group from a UDP-sugar to a small hydrophobic molecule. Khan *et al.* (2016) also used both physical (gamma radiation) and chemical (EMS) mutagens for mutagenesis using stevia leaf. The best mutagen doses were 0.4 % v/v EMS and 0.95 KR gamma radiation for selection of variants via direct shoot bud induction. On phytochemical analysis, the gamma mutated plants recorded twofold increase in rebaudioside A with lower stevioside content, whereas EMS mutated plants showed more than twofold increase in both stevioside and rebaudioside A as compared to control plants. These results were further confirmed by RT-PCR analysis of UGT74G1 and UGT76G1 genes that corresponds to stevioside and rebaudioside A biosynthesis, respectively. Gerami *et al.* (2017) assessed the improvement in glycoside content of stevia by EMS and reported that some properties of regenerated calli were influenced by different concentrations of EMS, different times of exposure and interactions of these two factors. One of the mutants recovered had the highest percentage of changes in the amount of stevioside (87.3%) and rebaudioside A (58.3%), respectively. Ahmad *et al*. (2019) assessed the effect of different concentrations of N-methyl-N-nitrosourea (MNU) in inducing mutation in Stevia seeds to produce genetic variations. Application of MNU reduced the germination percentage and germination rate of stevia seeds as compared to the control group. Prolonged exposure to the highest concentration of MNU recorded the lowest percentage of germination with lowest germination rate. Presence of seedlings with albino colour proved the mutagenic effect of MNU on Stevia genome and based on the percentage of seedlings with chlorophyll mutation, the most effective and efficient mutagenic treatment to induce mutation was recorded 60 min in 0.25 mM of MNU (Ahmad *et al*., 2019).

CONCLUSIONS

Stevia rebaudiana shows immense potential as an agricultural crop for the development of a high potency sweetener. The steviol glycosides, particularly rebaudioside A, extracted from the leaves of stevia has received great attention as a sugar substitute due to its most desirable sweetness and non-caloric nature. Safety studies conducted indicated the absence of any negative side effects so far after its consumption. High purity stevia extracts are approved for use as a sweetener worldwide. As stevia is self-incompatible with small seed size which showed reduced germination, therefore, development of new stevia genotypes with improved features is more suitable by mutation breeding using both physical and chemical mutagens. This has opened up the way for the development of a new stevia variety enriched with higher SGs, Reb-A which are suitable for more localized cultivation.

FUTURE PROSPECTS

Although the work of stevia crop improvement has been successfully running from past decades but there is still much room left for further varietal development for better quality and quantity of SGs and Reb-A. Use of several related germplasm sources through traditional breeding and also modern innovative approaches can play important role for both stevia plant ideotype development and to increase compounds responsible for sweetness. There is much scope to work

on consumers preferences as now much data available on the experiences of various consumers who have consume is in different forms available like dry leaves, leaves powder and stevia drops etc. recently use of nanoparticles in different crops has increased with the aim of character improvement of economic importance. The use of different nanoparticles has been done in stevia under *in vitro* conditions to improve germination, crop yield and content and quality of SGs and Reb-A. However, the absorption, translocation and accumulation mechanisms of various nanoparticles have not been properly studied and elucidated in stevia. Although the use of nanoparticles has been shown to have a plus impact on different plant characters but there is need to study the other effects with their transport, localization and translocation mechanism applied to the *in vitro* culture of stevia. These biotechnological tools along with use of various plant breeding approaches hold great promise for continued improvement in the plant characters of economic importance and to increase stevia production at commercial scale.

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