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GENETIC IMPROVEMENT OF *CENTELLA ASIATICA* L. FOR HIGH YIELD OF SAPONINS THROUGH COLCHICINE TREATMENT

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Abstract

Centella asiatica (Linn) Urban, is an important medicinal plants and commonly known as Brahmi or Mandookaparni belongs to the family Apiaceae. The present investigation was undertaken with a major objective of developing autotetraploid genotypes of Brahmi, employing the techniques of ploidy breeding, especially induction of autotetraploidy using colchicine, which is quick, reliable and very effective in high yield of saponins. *Centella asiatica* was used as experimental plant material and they treated of various concentration colchicine solution (0.1% to 1.0%) with different time durations (1to 8hrs.). I observed that, 0.2% colchicine concentrations with 4 hour durations were effective concentrations for inductions of autotetraploidy as compared to other. It confirm through morphological and anatomical characteristics of autotetrapoids and diploid plants. Then, *C. asiatica* estimation of spaonins of autotetraploidy was analyzed through HPLC method. It found that, the induced autotetraploids (colchiploids) showed 1.30 times high levels of medecassic acid, terminolic acid and asiatic acid (saponins) as compared to normal diploid plants. Thus the present investigation, establishes that it is possible to improve the quantity of therapeutically active compounds through polyploidy breeding by using colchicine treatment of medicinal plants.

Key Words: *Centella asiatica*, Colchicine, Autotetraploidy and Saponins

Introduction

Centella asiatica (Linn.) is a very important medicinal herb, traditionally used by ethnic people, as nervine tonic and for the treatment of asthma, hypertension, bronchitis, dropsy, skin diseases, and urethritis (Kakkar, 1988), since prehistoric times. *C. asiatica* has wound healing, anti-tumor, antibacterial, antifeedant, antituberculosis, antileprotic, and antioxidant properties (Chakraborty *et al*., 1996; Srivastava *et al*., 1997; Shakir *et al*., 2007). *Centella* is known to accumulate large amount of pentacyclic triterpenoid saponins which forms the major store house of secondary metabolites, providing active compounds stimulating cell rejuvenation, improving physical and mental health. Several studies point out that the major bioactive compounds in *Centella* are triterpenes (asiaticoside, asiatic acid, madecassoside and madecassic acid) and phenolic compounds particularly flavonoids (Farnsworth and Bunyapraphatsara, 1992; Inamdar *et al.*, 1996; Zainol *et al*., 2003).

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Sakshi *et al.,* (2010) reported that, as per the export and import bank of India *C.asiatica* is one of the important medicinal plants in the international market of medicinal plant trade. It also listed as Threatened plant species by the International Union for Conservation of Nature and Natural Resources (IUCN) and also as an endangered species. So, plant material of *C.asiatica* is an increasing demand international market day by day and therefore, there is an urgent need to genetically improve high yield of therapeutically active compound new plant. Induction of polyploidy is considering as a alternative technique for improvement and enhancement of the quality and quantity of important medicinal compound (Dhawan and Lavania, 1996). In this technique, colchicines play an important role in chromosome duplication in plants. Because, it is directly affect inhibiting the formation of microtubules and the polar migration of chromosomes. Therefore, artificial polyploidy plants are increased cell size due to increased complement of the chromosomes (Gao *et al*., 1996; Lavania, 1988), the phenomenon which may enhance the accumulation of commercially important

bioactive compounds. We are searched number of research paper published have reported that, increasing bioactive compound in medicinal plants through the induction polyploidy method.

Rawson (1944) reported an increase in the total alkaloid content in *Datura* due to induction of autotetraploidy. Similarly, Gottschalk (1976) has reported an increase in the content of the active substances (alkaloids) due to polyploidy induction in several plants. The content of alkaloid in autotetraploid plants of *Atropa belladonna* is 154% of that in the diploid plants (Jackson and Rowson, 1953). Olivera *et al*., (2004) has reported higher contents of stevioside in the autotetraploids of *Stevia rebaudiana* than diploid plants. In *Secutellaria baicalensis*, one tetraploid line exhibited an increase in baicalin of 4.6% (Gao *et al*., 2002). Tanavat *et al*., (2011) have reported the in vitro induction polyploidy in *C.asiatica* to increase of 11.00% in total triterpenes as compared to diploid. It observed that, not significant increasing bioactive compound in *Centella* in this method. Therefore, a major objective of the present investigation was to determine the appropriate method of colchicine application; finding out the effective concentration of colchicine and treatment duration for induction of autotetraploidy and quantitative estimation of saponins (HPLC) in the experimentally induced autotetraploids (colchiploids).

Materials and Methods

In the present study *Centella asiatica* was used as experimental plant material. *Centella* mother plants were obtained from Dhanwantari Udyan (Medicinal Garden) of Mahatma Phule Krishi Vidyapeeth, Rahuri, District-Ahmednagar, Maharashtra state, India. Then, collected plants were planted on well prepared seed beds containing mixture of fertile soil in green house and they were watered once daily by sprinkler. Six month old and acclimatized *Centella* plants were used for colchicine treatment.

In this experimental method different colchicine solutions (0.1% to 1.0%) were treated directly on immersion of running shoot tips with various time intervals (1 to 8hrs.) Ten plants running shoots were selected for each colchicines treatment and washed before treatments. Each treated plants were labeled properly and indicating the concentrations of the test solutions and duration of time (Jondhale and Apparao, 2015). Then, we observed variation in morphological and anatomical characteristics colchicine treated and untreated plants of *Centella*. The colchicine treated polyploidy plants were used for estimation of bioactive compound through HPLC method.

Plant Sample Preparation

Selected polyploidy and diploid leaves of the *Centella* were first washed with tap water and then rinsed with distilled water. After rinsed leaves were shade dried and shade dried mature leaves grind to obtain coarse powder. The grinded meal was sieved through a 56-mesh sieve to facilitate effective contact of the ruptured tissues and cells of the powder with the solvent used for extraction of biochemical compounds (Shah and Quadry, 2002).

Preparation of Samples for HPLC

The raw material 7gm of samples were weighed and transferred to 250 ml of beaker. The solution was extracted with 50ml of methanol by warming on water bath for about 20 min and then transfers the extract to 250 ml beaker. The procedure was repeated for 4-5 times till the raw material is completely extracted or till the extract is colorless.

Chemicals and Reagents of HPLC Analysis

Acetonitrite HPLC grade and Methanol was procured from Qualigens. Orthophosphoric acid (AR grade) was procured from Rankem. Water ultra-pure (18 MOhm resistance HPLC grade) was obtained from a sartorious water purification system. Reference standards of madecassoside, asiaticoside and asiatic acid were procured from R&D centre, NRPL, Bangalore.

Instrumentation and Chromatographic Conditions

High Performance Liquid Chromatographic system equipped with LC8A pump, SPD-M 10Avp Photo Array Director in combination with Class LC 10A software. Separation was achievedPhenyl reversed-phase column RP-18 (250×4.60mm, 5µm) .The mobile phase components were filtered through 0.45 µm membrane filter before use. Gradient elution was performed using, a mixture of Acetonitrile and buffer (Orthophosphoric acid in 1000ml of HPLC grade water). The flow rate was 1.8 ml/min and aliquots of 20µl were injected. The UV detection wavelength was set at 210 nm. The compounds were identified by comparing their retention times and UV spectra with those of the markers (Tiwari *et al*., 2010).

Result

In the present investigation, I observed that 0.2% colchicine solution with 4 hour duration treated *Centella* plants having effective concentration for induction polyploidy as compared to other concentration. Then, we observed variation in morphological and anatomical characteristics of colchicine treated (Polyploidy) and non

treated (Diploid) *Centella* and it also published in our previous paper (Jondhale and Apparao, 2011 and 2015). After, it is necessary to find out therapeutically active compounds of colchicine induced plants, because the main objective in induction of polyploidy is enhancement bioactive compounds in medicinal plants. Therefore, in this study yield of triterpenoid saponin compounds in colchiploids and diploid plants of *Centella asiatica* were estimated using the technique of High Performance Liquid Chromatography (HPLC). Compounds of these triterpenoid saponins (medecassic acid, terminolic acid, asiatic acid and asiaticoside) varied greatly in colchiploid and diploid plants of *C. asiatica.*

The HPLC analysis of diploid and autotetraploid *Centella* plants indicated that the colchicine induced autotetraploid plants showed higher levels of triterpenoid saponins as a whole as compared to diploid *C. asiatica* plants. Results revealed that the colchicine induced autotetraploid plants showed highest medecassic acid and terminolic acid (0.3 gm/gm dry wt.) contents which is 1.30 times (76.47%) higher than the diploids as compared to the untreated control plants. These results clearly indicate that the endogenous levels of medecassic acid and terminolic acid have gone up by 1.30 fold due to autotetraploidization.

HPLC analysis of control and colchicine induced autotetraploid plants revealed differences in their asiatic acid content Fig. 1 and 2. The asiatic acid content of the colchicine induced autotetraploid plants was 0.17 gm/gm dry wt., and that of control diploid plants was 0.06 gm/gm dry wt.. Thus, the autotetraploids produced more than double the quantity of asiatic acid (+283.33%) as compared to the corresponding normal diploid *C. asiatica*. These results also clearly indicate that the endogenous level of asiatic acid has 2.8 fold due to autotetraploidization.

The colchicine induced autotetraploid plants of *Centella* showed a non–significant increase of 3.2% in asiaticoside over that of the diploid *Centella asiatica* plants. The asiaticoside content was 0.92 gm/gm. dr. wt. in control plants where as it was 0.95 gm/gm dr.wt. in colchicine induced autotetraploids *Centella asiatica*. The diploid and colchicine induced autotetraploid plants did not show any significant difference in the contents of medecassoside and asiaticoside B contents Fig. 1 to 2.

Discussion

Thus, our experimental results clearly indicate that induction of autotetraploidy in *C. asiatica* is very affective in increasing the yield of therapeutically active compounds of this plant. Similar increase in the yield of therapeutically active compounds, as a result of induction of autotetraploidy, was also reported by several authors but in different medicinal plants. In *Centella asiatica* was reported to increase 11.00% total triterpenes yield as compared to diploid plants by using *in vitro* induction polyploidy method (Tanavat *et al.,* 2011). But, it also reported that there is not significant increase in total triterpenes as compared to diploid plants. In our result is clearly mentioned that, significantly increase yield of

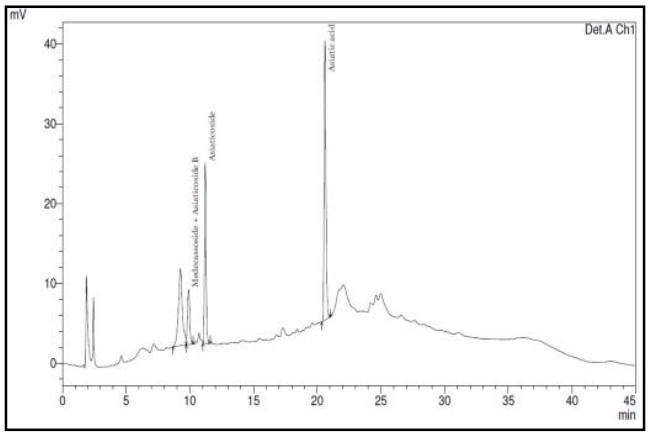

Fig. 3: HPLC Chromatogram of the standard used for the quantitative determination of Saponins.

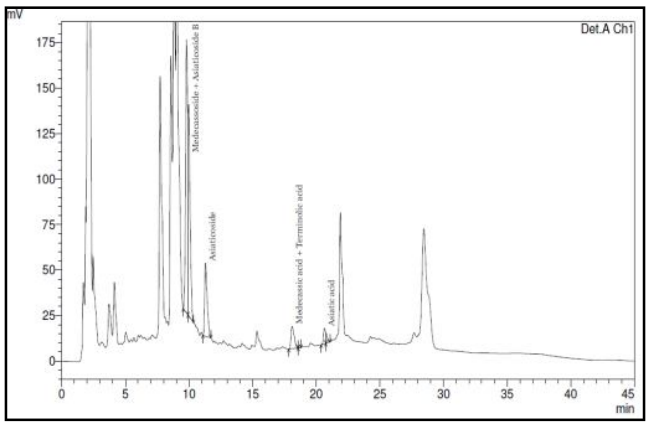

Fig. 1: HPLC Chromatogram of the control plant extracts of *Centella asiatica* used for the quantitative determination of Saponins.

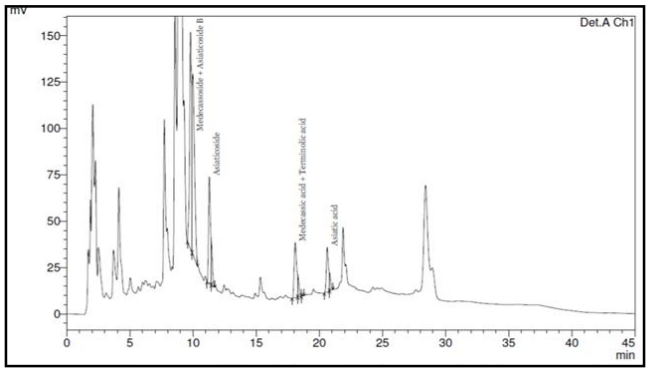

Fig. 2: HPLC Chromatogram of the Colchicine Induced Autotetraploid Plant Extracts of *Centella asiatica* used for the Quantitative Determination of Saponins.

saponins as compared to normal diploid plant by using induction of polyploidy method. Rawson (1944) reported an increase in the total alkaloid content in *Datura* due to induction of autotetraploidy. Bhatt and Heble (1978) also reported an increase in solasodine content in fruits of a spiny *Datura* due to induction of autotetraploidy. Krishnan (1998) reported that induced autotetraploids of *Solanum viarum* are characterized by higher solasodine content which was up to 50% higher than diploid. Recently, Phithak and Kawee (2019) reported that, maximum yield total bacoside content (1.55 mg) was obtained from an autotetraploid plant, which was 2.3 fold higher than the level in diploid plants.

It has been reported that the tetraploid plants of *Hyoscyamus muticus*, had nearly 1.5 times higher economic production potential and compared with that of its diploid counterparts (Lavania, 1988). In addition, the leaf, stem and root which can be useful parts in most medicinal plants are usually bigger in polyploids. Thus, the polyploids may increase biomass or product yields (Gao *et al*., 1996). Induction of artificial polyploidy is useful in increasing the production of important medicinal compounds (Dhawan and Lavania, 1996). The autotetraploids obtained in the present investigation may prove useful and open new possibility for genetic breeding programme of *C. asiatica* since polyploid individuals have higher content of asiaticoside, medecassic acid, terminolic acid and asiatic acid than the wild diploid plants.

Conclusion

In the present study, I observed that, colchicine is best and effective chemical to induction of autopolyploidy in not only *Centella* but also all medicinal plants. The HPLC method is one of the effective methods to estimation of bioactive compounds in medicinal plants. Finally, I concluded that, colchicine induced *C.asiatica* autopolyploids having enhancement of therapeutically active compound as compared normal diploid plants. It found that saponins content (asiaticoside, medecassic acid, terminolic acid and asiatic acid) of *C.asiatica* polyploidy plants was significantly higher than of the normal diploid plants.

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