The Bioscan 10(2): 609-611, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

IMPACT OF STEVIA (*STEVIA REBAUDIANA* BERT.) POLYPLODIZATION ON LEAF YIELD AND ATTRIBUTES

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KEYWORDS
Mixaploid
Polyploids
Stevia
Stevia rebaudiana

Received on :
12.01.2015

Accepted on :
26.04.2015

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ABSTRACT

A field experiment was carried out at Department of Horticulture, University of Agricultural Sciences, Bangalore (Karnataka) during 2013-14 to evaluate stevia (*Stevia rebaudiana* Bert.) polyploids for higher biomass and to work out the cost of cultivation. The experiment was laid out by adapting Randomized Complete Block Design with ten treatments and three replications. The treatment included nine induced polyploids of stevia with varied ploidy levels and one untreated diploid. Significant differences were observed with respect to yielding ability of the plant. At harvest the yield parameters *viz.*,leaf thickness (0.57 mm), leaf area (15.00 cm^2), fresh weight of leaves (126.63 g/ plant), dry weight of leaves (32.90 g/ plant) were maximum in mixaploid, with 93.99% increase in yield compare to normal diploids. From the present investigation it can be conclude that, yield performance of all induced polyploids were better compare to diploid. Among the polyploids mixaploid shown best results with respect to yielding ability.

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a perennial herb belonging to Asteraceae family and native of Paraguay. It is often referred to as sweet herb of Paraguay. Leaves of stevia produce diterpene glycosides (stevioside and rebaudioside), which are high-potency sweeteners (300 times sweeter than sucrose) and substitutes for sugar (Abdullateef and Osman, 2011), can be used for treatment of diabetes, obesity, hypertension and dental caries prevention (Jaroslav *et al.*, 2006). All species of stevia were diploid with 2n=22 chromosomes. The basic chromosome number is x=11 (Frederico *et al.*, 1996). The plant can be cultivated under semi-humid subtropicalclimate, shows higher herb production under high light intensity and warm temperature. The first harvest of the crop can be obtained in four months after planting and subsequent harvest once in every 3 months. The whole plant is harvested above the ground level, followed by drying and threshing (Brandle *et al.*, 1998). The total life span of the crop is reported to be 7-8 years with economical crop duration of four years. The quantity of dry leaves that can be harvested varies from 15 to 35 g per plant per harvest (Mishra *et al.*, 2010).

Because of the less yield in present cultivars, the crop is not considered economical for cultivation. The yield can be improved various crop improvement programs. Production of larger leaves and other plant parts can be accomplished by induction of polyploidy (Yadav *et al.*, 2013). Manipulation of ploidy is a valuable tool used in plant breeding programmes to increase the organ size, improve agronomic yield (Blakeslee and Avery, 1937). Leaves are the economic part in stevia, to increase the size of the leaves and biomass production, polyploids were developed in stevia at Department of Horticulture, UAS, Bangalore by using different concentration of colchicines. The ploidy level was confirmed through flow-cytometry by Suhad (2012). The morphological analysis was carried out and variability was observed in polyploids compare to untreated diploid (Chavan, 2013). To check the yield performance of these induced polyploids an evaluation study was taken under field condition with an objective of screening high yielding polyploids.

MATERIALS AND METHODS

Experimental site and Treatments

Experiment was conducted during 2013-2014 under field condition at Sugandhavana, Department of Horticulture, University of Agricultural Sciences, Bangalore, which is situated at 12°58′ latitude North, 77°11′ longitude East and altitude about 930 m above MSL. The experiment was laid out in Randomized Complete Block Design (RCBD) with ten treatments and three replications. Treatment consisted of nine induced polyploid plants of different levels of ploidy with one untreated diploid (2n=22).

Treatment details:Treatment includes induced polyploids and control.

T_1: Diploid (untreated)

T_2: Diploid (treated with 0.25 per cent Colchicine)

T_3: Diploid (treated with 1.00 per cent Colchicine)

T_4: Diploid (treated with 2.50 per cent Colchicine)

T_5: Triploid (treated with 1.50 per cent Colchicine)

T_6: Triploid (treated with 2.00 per cent Colchicine)

T_7: Triploid (treated with 2.50 per cent Colchicine)

T_8: Mixaploid (treated with 0.50 per cent Colchicine)

T_9: Tetraploid (treated with 1.00 per cent Colchicine)

T_{10}: Tetraploid (treated with 1.50 per cent Colchicine)

Well rooted cuttings of different treatments were planted in separate blocks with a spacing of 45X 22.5 cm. The gross plot size was 1.65x 2.55 m with a net plot size of 2.25 × 1.35 m. A fertilizer dose of 70:35:45 kg NPK/ ha (Vasanthi *et al.*, 2011) and FYM of 15 t/ hawere applied before planting and a light irrigation was provided immediately after planting. Subsequent irrigation was provided at weekly intervals depending on the soil moisture status. Crop management and cultural practices were taken up at regular intervals like flower removal, weeding, earthing up, top dressing *etc*.

Yield and yield attributes

The plants were observed every day and number of days when 50% of the plants starts flowering in treatment unit was recorded. Crop was harvested after four months and observations were recorded which focused on leaf thickness, leaf area, leaf fresh weight, fresh weight of stems, leaf dry weight. The observations were taken from ten selected plants in each replication and the mean was recorded. Leaf area was measured by using the leaf area meter and leaf thickness was recorded using digital vernier calipers. A moderate sized leaf was sand witched between two glass slides and the thickness was measured and the leaf thickness was obtained by deducting the glass slide thickness from the total thickness(Chavan *et al.*, 2014). For recording the fresh leaf yield, the plants were cut down at the base and leaves are separated from the stem, weighed on electronic balance. To obtain the dry weight, leaves were shade dried at room temperature for 4 days and weighed with an electronic balance. Drying percentage of leaf was calculated by dividing fresh weight from the dry weight and multiplied by 100. Percent increase in yield was calculated by dividing yield of control treatment from increased yield and multiplying by 100.

RESULTS AND DISCUSSION

Polyploidy often generates variants that may possess useful characteristic and by doubling the gene products. Ploidy manipulation is considered as a valuable tool in genetic improvement of many plants (Thao *et al.*, 2003). In the present investigation, it was observed by comparing all the treatments that, ploidy level has a positive influence on yield parameters. Significant differences were observed in yield parameters such as leaf thickness, leaf area, fresh leaf weight and dry leaf weight in stevia as presented in Table 1.

Delayed flowering was recorded in Mixaploid (73.3 days for 50% flowering), but it was on par with T6 (69.67 days), T7 (69 days) and T9 (66 days) and T10 (63.33 days) compare to other diploids and early flowering was recorded in control (39.67 days).Mixaploid (T8) recorded the maximum leaf thickness (0.57 mm) and leaf area (15.00 cm2), which was on par with Tetraploid (T10). Minimum leaf area (9.36 cm2) and minimum leaf thickness (0.35 mm) was recorded in control plants. The present findings were in agreement with previous study conducted by Chavan *et al.* (2014).Production of larger leaves and other plant parts was due to an increase in organ size by induction of polyploidy. The results corroborate with the findings of Ramesh *et al.* (2011) in mulberry that, leaf area and leaf yield increased considerably in colchicine induced plants, compared to control. The main aim of stevia breeding is to increase the leaf yield as like in mulberry. The leaf area and thickness was considerably increased in polyploid populations.

The maximum average fresh leaf weight (126.63 g)was found in the mixaploid (T8), but it was statistically at par with Tetraploid (T10), Triploid- (T6), (T7) and (T5).The mixaploid also recorded maximumdry weight of leaves per plant (32.90 g). The minimum average fresh leaf weight (65.27 g) and minimum average dry leaf weight per plant (16.96 g) was recorded in control (T1). The dry weight depends on the total fresh weight and moisture content present in the leaves. Fresh weight of leaf is directly influenced by leaf area, leaf thickness and number of leaves etc. Because of an increase in the yield assisting attributes in polyploids, a corresponding increase in plant biomass can be expected. Yields are expressed in terms

Table 1: Parameters influencing yield in *Stevia rebaudiana* polyploids

Treatments	Days to 50% Flowering	Leaf thickness (mm)	Leaf area (cm^2)	Herb yield (g/ plant) Fresh wt.	Dry wt.	Dry recovery (%)
T_1- Diploid (untreated control)	39.67	0.35	9.36	65.27	16.96	26.06
T_2- Diploid (Colchicine 0.25%)	52.33	0.48	11.00	109.00	28.67*	26.39
T_3- Diploid (Colchicine 1.00%)	62.67	0.47	11.20	117.30*	31.47*	26.79
T_4- Diploid (Colchicine 2.50%)	53.67	0.47	12.17	101.07	27.10	26.93
T_5- Triploid (Colchicine 1.50%)	62.00	0.52	11.77	119.00*	29.43*	24.86
T_6- Triploid (Colchicine 2.00%)	69.67*	0.49	12.13	124.33*	26.43	21.64
T_7- Triploid (Colchicine 2.50%)	69.00	0.52	13.43	120.67*	31.77*	26.74
T_8- Mixaploid (Colchicine 0.50%)	73.33*	0.57*	15.00*	126.63*	32.90*	26.08
T_9-Tetraploid (Colchicine 1.00%)	66.00*	0.52	14.03*	93.42	27.57	29.71
T_{10}-Tetraploid (Colchicine 1.50%)	63.33*	0.56*	14.13*	121.20*	32.08*	26.41
Mean	61.17	0.49	12.42	109.79	28.44	26.22
S. Em ±	3.41	0.01	0.39	5.21	1.63	1.32
CD (p=0.05)	10.13	0.03	1.15	15.48	4.85	NS

*Significant at 5 % level or CD (p=0.05)

Table 2: Yield parameters of *Stevia rebaudiana* polyploids

Treatments	Dry leaf weight (g/ plant)	% increase in yield compare to control	Dry leaf yield (single harvest)		Dry leaf yield per year(kg/ ha)
			(g/ plot)	(kg/ ha)	
T_1- Diploid (untreated control)	16.96	-	508.7	1209.03	4836.13
T_2- Diploid (Colchicine 0.25%)	28.67	69.04	860	2043.97	8175.88
T_3- Diploid (Colchicine 1.00%)	31.47	85.55	944	2243.61	8974.45
T_4- Diploid (Colchicine 2.50%)	27.10	59.79	813	1932.26	7729.06
T_5- Triploid (Colchicine 1.50%)	29.43	73.53	883	2098.63	8394.53
T_6- Triploid (Colchicine 2.00%)	26.43	55.84	793	1884.73	7538.92
T_7- Triploid (Colchicine 2.50%)	31.77	87.32	953	2265.00	9060.01
T_8- Mixaploid (Colchicine 0.50%)	32.90	93.99	987	2345.81	9383.24
T_9-Tetraploid (Colchicine 1.00%)	27.57	62.56	827	1965.54	7862.15
T_{10}-Tetraploid (Colchicine 1.50%)	32.08	89.15	962.3	2287.11	9148.43
S. Em ±	1.63		48.97	116.40	465.59
CD (p=0.05)	4.85		145.51	345.83	1383.34

Note: Four harvests were taken in a year,

of kg/ha and presented in Table 2. Delayed flowering in mixaploid leads plants to remain in vegetative stage and enhance the leaf yield. Polyploidy is often induced in plants of economical interest, in order to produce variability that can improve the yield. Shaopan *et al.* (1995), Yadav *et al.* (2013) and Oliveira *et al.* (2004) revealed that, tetraploid stevia has larger leaf size, thickness and have potentiality to increase the biomass in comparison with diploid strains. Moreover characteristics of mixaploid stevia plant are similar to tetraploids. The present findings are in conformity with the observation of Valois (1992) in stevia. The report of Gandhi and Patil (1997) also confirms that, induction of polyploidy to improve agronomic yields is a process commonly used in plants of economic interest and has been applied to many plant species.

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