DOI : 10.5958/0974-0112.2015.00084.5

Indian J. Hort. 72(3), September 2015: 426-428

Short communication

Development of colchitetraploids with improved fertility in watermelon

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ABSTRACT

Tetraploid plants were produced in the watermelon cultivar 'Sugar Baby' through seedling treatment with colchicine solution (0.5%). Path coefficient analysis was conducted to find out the relative contribution of selected characters towards number of seeds across C1, C2 and C3 generations of tetraploid population originated from 0.5% colchicine treatment. Direct effect of number of chloroplasts per guard cell was found highly significant for seed number (r = 0.269). Among other characters days to emergence of male (r = -0.307) and female flower (r = -0.259) and gourd cell width (r = -0.33) exhibited highly significant negative direct effect on seed number. Tetraploids could be improved for seed number by selecting the plants in each generation with high chloroplasts per guard cell (>22), earliness to form male flower (<48 days), female flower (<53) and low gourd cell width (<22 μm).

Keywords: Watermelon, colchitetraploid, seed number.

Seedlessness is an important and desirable breeding objective in watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai]. Seedless watermelons are triploids (3x = 33) and result from crossing a tetraploid (2n = 4x = 44) seed parent with a diploid (2n = 22) pollen parent. Breeding stable tetraploid lines with adequate fertility is a major challenge in triploid watermelon production (Mohr, 7). Tetraploid plants exhibit low fertility in terms of seed number and generally require at least 8-10 years of self-pollination before enough plants are obtained for commercial triploid seed production (Compton and Gray, 3). Traditionally, tetraploid lines for hybridization have been obtained by treating newly emerged diploid seedlings with colchicine concentration ranging from 0.2 to 1.0% (Suying *et al*., 9). However, this treatment produces a limited number of tetraploids and mostly chimeric seedlings that possess vines of mixed ploidy (Compton *et al*., 1; Jaskani *et al*., 5). A stable tetraploid can be isolated through appropriating the initial concentration of colchicine ranging from 0.1% under *in vivo* and applying early selection based on traits linked to fertility. Fertility of tetraploids can be improved by selecting plants with higher seed number in successive generation. Hence, an investigation was carried out to induce polyploidy in the commercial watermelon cultivar 'Sugar Baby' using colchicine and the resultant C1, C2 and C3 generations were morphologically characterized. The aim of the present investigation was to develop an efficient selection system for the early detection of tetraploids with improved fertility in watermelon.

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Polyploidy was induced in watermelon cultivar 'Sugar Baby' through seed and seedling treatment with colchicine solution. Experiment was undertaken at Department of Olericulture, College of Horticulture, KAU, Thrissur, India during the period 2009-2012. Seeds of 'Sugar Baby' were soaked in clean water for 6 h and then soaked in colchicine solution (0.1, 0.5, 1, 1.5, 2.0%) for 24 h. The seeds were rinsed for 30 seconds before sowing, sown in polybags filled with sand and screened for ploidy. For seedling treatment, seeds were sown in polyhouse. Once seedlings emerged, a drop of colchicine (0.1 and 0.5%) was added to the shoot apex between the cotyledons. The chemical solution was applied to the growing point during morning hours for three consecutive days. The ploidy evaluation of seedlings was made by counting number of chloroplasts in each guard cell of stomata at 3-5 true leaves emergence stage (Sari *et al*., 8).

Selfed seeds from polyploid plants developed by 0.5% seedling treatment were sown for raising C2 generation. All the observations described above were taken from randomly selected 22 plants in C2 generation. The plants in C2 generation were further selfed and those yielding more than 100 seeds per fruit were selected for raising C3 generation. Observations detailed above were recorded from randomly selected 32 plants in C3 generation. The data generated were subjected to Analysis of Variance (ANOVA) and least significant test. Correlation coefficients were worked out as suggested by Wright (10) and Dewey and Lu (4). Path coefficient analysis was conducted to find out the measure of direct and indirect effects of any one variable on seed number. All computations were done by using SPAR2 software for Windows®.

Watermelon seeds failed to germinate after being treated with 1, 1.5 and 2% colchicine and could not be included for recording observation. Significant difference were observed for different characters treated with colchicines at three different concentrations and plants generated from 0.5% colchicine seedling treatment produced giant guard cells with maximum number (22) of chloroplasts per guard cell (Table 1). These plants produced large sized pollen grain and took more number of days for the emergence of male (58.33) and female flowers (64.93). The number of seeds was found to be least in plants developed from seedlings treated with 0.5% colchicine (28.25). In general, slower growth rate and delayed appearance of shoots and flowers were observed in all colchicine treated seedlings compared to that in control. Higher concentration of colchicine (0.6%) had a damaging influence in polyploidy induction (Compton *et al.*, 2).

Colchicine seedling treatment was found to be more effective than seed treatment in inducing polyploidy. Similar result is reported from China, where seedling treatment with 0.3 and 0.4% colchicine induced tetraploidy in yellow fleshed watermelon (Zhang *et al.*, 11). Frequency of chloroplasts in guard cell is an efficient indicator of the ploidy level (Jaskani *et al.*, 5). Microscopic examination of stomata proved the utility of chloroplast number and guard cell dimension for screening ploidy level in watermelon and could be helpful in successive generations to develop tetraploid inbred. The number of chloroplasts in seedlings treated with 0.5% colchicine was almost double (22) and the seeds from this treatment was advanced to C2 and C3 generation.

Path coefficient analysis was conducted to find out the relative contribution of selected characters towards number of seeds across C1, C2 and C3 generations of tetraploid population originated from 0.5% seedling treatment (Table 2). Direct effect of number of chloroplasts per guard cell was found highly significant for seed number (r = 0.269). Among other characters, days to emergence of male (r = -0.307) and female flower (r = -0.259) and gourd cell width (r = -0.33) exhibited highly significant negative direct effect on seed number. Size of pollen grain or gourd cell length exhibited no significant direct or indirect effect in deciding seed number.

The strong, positive correlation (r = 0.226) between number of chloroplasts per guard cell and seed number per fruit resulted from the strong direct effect (r = 0.26) of chloroplasts per guard cell and though fruit weight expressed significant positive correlation with seed number, direct or indirect effect was not significant. High negative correlation between days to emergence of male and female flower as well as their high direct effect on seed number shows the scope of improving seed number by selecting early flowering genotypes in advanced generations. The characters, *viz.*, number of chloroplasts per gourd cell, earliness and gourd cell width are not indirectly effected by other characters and exhibited high direct effect on seed number and hence can be taken as a reliable character for selecting the tetraploids with increased seed number (>100) in advanced generations.

Reduced fertility is an inherent drawback of induced autotetraploids, which has been attributed to meiotic abnormalities and physio-genetic imbalance. Optimising the colchicine dosage and exercising selection for seed set in subsequent generations of colchitetraploids has resulted in fertility improvement in certain crop plants (Kumar *et al.*, 7). It can be concluded that polyploids generated through 0.5% colchicine seedling treatment could be improved for fertility in terms of higher seed number by selecting the plants in each generation with high chloroplasts per guard cell (> 22), earliness to form male flower

Table 1. Characterization of colchicine treated population during C1 generation.

Character	0.1% (seed treatment)	0.1% seedling	0.5% seedling	Control	LSD (p = 0.05)
No. of chloroplasts per guard cell	18.75	17.25	22.00	12.00	0.754
Guard cell length (µm)	28.71	28.16	28.46	20.57	2.682
Guard cell width (µm)	22.39	21.46	22.18	15.51	1.47
Pollen size (µm)	34.415	32.037	37.225	31.635	2.684
Days for the emergence of male flower	54.5	56.75	58.33	29.0	5.594
Days for the emergence of female flower	69.0	62.5	64.93	32.0	3.445
Fruit wt. (kg)	1.715	2.637	0.770	3.575	1.438
Seed No.	151	445	28.25	529.0	211

Table 2. Direct and Indirect effect of selected characters on seed number across C1, C2 and C3 generations (pooled value) of tetraploid population.

Trait	Chloroplast/ guard cell	Size of pollen grain	Fruit wt.	Days to emergence of male flower	Days to emergence of female flower	Guard cell length	Guard cell width	Total correlation
Chloroplast/guard cell	**0.2639****	0.0343	-0.0097	-0.0643	-0.0281	-0.0393	-0.0289	0.227
Size of pollen grain	-0.0631	**-0.0891**	-0.0201	0.0279	-0.0328	0.0074	-0.0286	-0.19
Fruit wt.	-0.0186	0.0210	**0.0855**	0.0719	0.0842	0.0045	0.1376	0.38
Days to emergence of male flower	0.0343	0.0081	-0.0200	**-0.3071****	-0.1203	-0.0340	-0.0207	-0.45
Days to emergence of female flower	0.0178	-0.0113	-0.0278	-0.1424	**-0.2594***	-0.0296	-0.1273	-0.57
Guard cell length	-0.0652	-0.0067	0.0039	0.1057	0.0778	**0.0987**	-0.0181	0.19
Guard cell width	0.0141	-0.0076	-0.0350	-0.0189	-0.0981	0.0053	**-0.3365****	-0.47

Residual = 0.4734

(< 48 days), female flower (< 53 days) and low gourd cell width (< 22 µm). Selfing the selected plants with optimum values for these characters over the population mean is effective for the advancement of generation producing tetraploid plants with higher fertility in watermelon.

ACKNOWLEDGEMENT

Authors are grateful to the Kerala State Council for Science Technology and Environment, Thiruvananthapuram for providing financial assistance.

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Received : April, 2013; Revised : June, 2015; Accepted : August, 2015