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Genetic Manipulation of Some Cassava Varieties Using Varying Levels of Colchicine2

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Abstract
Some high yielding varieties of cassava can be very recalcitrant to flowering. As a result, become very difficult for recombination to take place. In order to address this challenge, five high yielding cassava varieties (TMS98/0505, TMS94/4479, TMS94/1632, TMS92/0057, and TMS98/0581) treated with three levels of colchicine (0, 2 and 4)ppm, were planted in a 4m x 5m plot size, at a spacing of 1mx1m in a randomized complete block design replicated three times; with the aim to improve on the architecture, flowering and capsule production at National Root Crops Research Institute, Umudike. The photosynthetic efficiency of the materials was measured using plant photosynthetic Efficient Analyzer and the result showed significant differences at ($p<0.05$) among varieties with TMS96/4779 as the highest (0.156). The result of the analysis of variance in the branching habits, inflorescences development and capsule production showed significant differences. There were significant differences in the number of inflorescences produced by different branch levels and the third branch (15.86cm) with the best treatment consecration of 2ppm'. The best early Variety TMS 98/0505 gave the highest branch height at each branch level with the first branch level of 6.78cm, second branch level of 14.00cm inflorescence production of (2.96) on the first branch level was found on variety TMS98/1632, while the third level of variety TMS98/0505 gave the highest number of capsules at concentration 2 ppm. This study showed that colchicine has positive significance on the induction of flowering, inflorescence and capsule production.

Keywords: Colchicine, induced mutation, capsules, inflorescences and architecture

Introduction

In order to generate a wide range of variables for diverse purposes and usefulness in cassava, there is need to overcome the bottle neck of conventional breeding and create room for the development of those traits that naturally do not exist or have been lost through genetic erosion (Ceballos, 2004); (Hatten,2013). Improvement of cassava through conventional method is limited by many factors ranging from the genetic boundary (monoecious and protogynous), planting time, branching habit, and prevailing environmental conditions (Byrne, 1984; Ceballos *et al.,* 2002). Therefore, technologies to increase flowering and botanical seed production have to be diversified (Fregene *et al.,* 2003). Imminent among them is the development of cassava varieties with good photosynthetic efficiency and good plant architecture that can enhance flowering, capsule production, seed production and thereby increasing the chances of recombination of characters, developing specific and desired qualities. Branching in cassava is typically dichotomous or trichotomous (Alves *et al*., 2020) with branchlets that run in levels, having at the branching point a terminal inflorescence. Flower bud formation is preceded by apical branching or forking which is a prominent visual indication of commencement of flowering. A flower bud typically forms when the plant branches, so that more highly branched genotypes are more prolific than those with a sparsely branched habit. Although farmers generally prefer the non-branching cassava type because it facilitates cultivation practices, their usefulness as progenitors in breeding is limited by their low seed production. Byrne and Gracen (1982). Flowering is strongly influenced by prevailing environmental conditions, specific genotypes and time of planting which may vary from a few to more than 24 months (Byrne, 1984). Hybridization and selection have been exploited (Manyong *et al.*, 2000), but could work only within the inter-specific and intra-generic boundary of the crop, low genetic base, regular genetic erosion, irregular flowering and time consuming common with root and tuber crops breeding Marcela et., al (l2000). Mutation breeding using chemical means such as flowering hormone has been identified to be effective in improving branching, enhancing genetic variation in seed production and improvement of vegetatively propagated crops such as root and tuber crops. The principal aim of modern plant breeding is to

produce desired varieties in the shortest possible time so as to meet the immediate need for cassava and its products. This work, therefore, calls for the development and use of alternative technological tools that will move cassava production to a sustainable height. This paper, therefore, reports on the use of varying levels of flowering hormone "colchicine" to enhance branching, flowering and seed production in cassava.

Materials and Methods

The experiment was conducted at National Root Crops Research Institute (NRCRI), Umudike Research farm in Southeastern Nigeria. In 2018, 25cm stacks of five cassava varieties were treated with mutation-inducing hormone colchicine at three different levels –0ppm, 2ppm, and 4ppm. They were soaked in the solution for 30 minutes, air-dried for 24 hours, and pre-sprouted in the nursery bags in the screen house to generate seedlings for fieldwork. Three weeks after, the seedlings were transplanted in the field in a randomized complete block design. Seven months after planting, twenty-five pieces of 25cm stake cuttings of each of the cassava varies were cut from the mature plants raised from the treated materials and planted in a well harrowed and ridged field in a 4mx5m randomized complete block design, at a spacing of 1m x1m intra and inter-row replicated three times. Post and pre-emergence herbicides were applied immediately after planting and the field was maintained by applying 15; 15;15 NPK fertilizer. All the other agronomic practices were followed. The photosynthetic efficiency of the materials was measured at harvest using plant Efficient Analyzer (PEA). While other data were collected on their morphological features by counting the branching levels, inflorescences number and number of capsules produced. Data generated were subjected to analysis of variance (ANOVA) to test significant variation among the mean values using Genstat statistical package (2007) edition and means were separated using Duncan multiple range Test

Results and Discussion

The statistical analyses carried out on the physiological features such as Photosynthetic efficiency potential, branching level, inflorescence number and capsule number showed significant variation in the different features evaluated. Table1: showed that there was significant at ($p<0.05$) difference in the photosynthetic efficiency potential of the different varieties. The photosynthetic efficiency potential ranged from 0.0049 to0.156, while the highest value was produced by variety TMS94/4779 followed by Variety TMS92/0057 with (0.0082) value and TMS98/ 0505 with (0.0068 value), while Variety TMS94/1632 had the least value of0.0049. According to (Kawano, 2003). Photosynthetic efficiency is one of the Physiological traits that serve as indirect selection criteria to improve cassava, especially its yield potential and stability and should be combined with other yield related traits. This result shows that different cassava varieties have different Photosynthetic ability which can increase yield according to Long, (2005) who confirmed that an increase in Photosynthetic ability increase Carbon dioxide uptake and yield in turn.

Effect of colchicine on the level of branches

Table 2 showed the effect of colchicine on the branching level of cassava. The result in the table showed that there was a high significant difference at ($p<0.05$) across the varieties of branch height. Variety TMS 98/0505 had the highest branch height at first level of branching (7.55), second level of branching level (15.22), and the third level of branching (17 .22) at the 4 ppm concentration, while the least branch height was found on variety TMS 92/0057 at 0ppm concentration with 0.56cm branch height on first level of branching, 3.33cm on second level of branching and 0.78cm on the third level of branching. This result was in contrast with (Zhang *et al,* 2010) who reported shorter mean height for colchicine treated materials.

Effect of colchicine on the inflorescence production

Table 3 below, shows the effect of colchicine on the number of Inflorescence production. The result showed that the highest Inflorescence production was found on the third branch level with a range of 1.56 to 29.11. The highest number of Inflorescence 29.11 was found on varieties TMS92/0057 at a concentration level 2ppm, while the least number of Inflorescence was found on the control 0ppm without any of the Inflorescence (0.0). This result, therefore, showed that the highest dosage of colchicine influenced more Inflorescence production than the lower dosage. This result is in line with Zhang *et al.* (2010) and Ye *et al*. (2010) who reported that the use of flowering hormone enhances branching and fruiting.

Effect of Colchicine on Capsule Production

The effect of colchicine on capsule production was shown in Table 4. The result showed that the capsule on the first branching level was scanty and few. The result also showed that the highest capsule production was found on the third branch level with a range of 0.00 to 34.33. The highest number of capsules 34.33 was found on variety TMS98/0505 at Concentration level 2ppm while the least number of capsules was found on variety TMS 92/0057.

Conclusion

Prolific branching is needed in cassava breeding to enhance flowering, inflorescence and capsule production to develop a large population for selection and this study has proven that this can be possible through the use of colchicine. This study also revealed that Concentration is an important point to consider as levels of concentrations affected the number of plants that flowered, the number of inflorescences, branch length and seed production. The concentration of 4ppm favoured branch height elongation while 2ppm concentration support the high number of inflorescence production. This finding could be recommended to be used in other crops that are recalcitrant to flowering and fruiting production and colchicine can be used to

enhance flowering in other crops that have low flowering ability.

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Table 1: Evaluation of photosynthetic Efficiency and concentration on the cassava varieties used

Varieties	Photosynthetic efficacy potential
TMS94/1632	0.0049[c]
TMS98/0505	0.0068[ab]
TMS92/0057	0.0082[ab]
TMS92/4779	0.156[a]
Concentration	
0ppm	0.0099[a]
2ppm	0.0101[a]
4ppm	0.0066[a]

Table 2: Effect of colchicine on the branching level of some cassava varieties

Genotype Conc.	Br1(cm)	Br2(cm)	Br3(cm)
TMS92/0057 0ppm			
	0.56[c]	3.33[e]	0.78[b]
2ppm	3.22[c]	4.80[de]	1.89[b]
4ppm	3.33[c]	6.22[de]	2.11[b]
TMS98/1632			
0ppm	4.33[ab]	7.11[de]	2.33[ab]
2ppm	3.11[abc]	9.78[de]	9.52[ab]
4ppm	3.78[c]	7.33[de]	7.22[ab]
TMS94/4779			
0ppm	4.55[ab]	8.33[cde]	6.55[ab]
2ppm	3.78[abc]	4.22[e]	9.55[ab]
4ppm	4.33[c]	7.00$_{de}$	9.78[ab]
TMS98/0505			
0ppm	5.66[c]	5.66b[c]	14.88[a]
2ppm	7.11[ab]	*15.22[a]	*17.22[a]
4ppm	*7.55[a]	14.22[db]	15.44[a]

Key: Br1, Br2 and Br3 = First, Second and Third branching levels

Table 3: Effect of colchicine on the inflorescence production in cassava

Genotype (ppm)	Concentration	Number of inflorescences		
		First branching	Second branching	Third branching
TMS92/0057	0	0.00[a]	0.00[d]	0.00[c]
	2	0.00[a]	0.66[ab]	1.56[c]
	4	0.67[a]	2.67[ab]	3.33[c]
TMS98/1632	0	1.88[a]	5.33[abc]	9.11[ab]
	2	2.00[a]	7.33[abc]	13.37[b]
	4	5.00[a]	6.11[abd]	11.89[ab]
TMS94/4779	1	0.00[a]	1.44[cd]	1.78[c]
	2	0.00[a]	2.54b[cd]	9.44[bc]
	4	0.78[a]	3.50[bcd]	11.89[ab]
TMS98/0505	0	1.44[b]	11.66[a]	11.44[a]
	2	4.44[a]	12.00[a]	29.11[a]
	4	0.66[a]	9.56[ab]	23.78[ab]

Table 4: Effect of colchicine on the capsule production in cassava

Genotype (ppm)	Concentration	Number of inflorescences		
		First branching	Second branching	Third branching
TMS92/0057	0	0.00[a]	0.44c	0.00[c]
	2	0.00[a]	0.00c	0.00c
	4	0.0.44[a]	1.78c	3.89[c]
TMS98/1632	0	2.33a	3.11b	4.33c
	2	0.67b	5.44a	13.37[b]
	4	2.89a	3.44b	4.77c
TMS94/4779	1	0.00[a]	0.00c	0.00c
	2	0.00[a]	0.00c	1.22c
	4	0.00a	1.33c	0.00c
TMS98/0505	0	0.00a	6.11ab	20.44[ab]
	2	6.89a	10.66a	33.44[a]
	4	1.11b	3.22b	22.33[ab]