Potential Banana cv 'Lakatan' Somaclones Induced by Long Culture Period and High 2,4-D Concentration

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The study was undertaken to determine the effect of long subculture and high dosage of 2,4-D on the yield and other postharvest traits of banana cv ‘Lakatan’ somaclones. Morphological evaluation was done on 2,040 plants (planted in a 2.5ha field (3x3m distance of planting, laid in Randomized Complete Block Design (RCBD) in factorial arrangement) using the International Network for the Improvement of Banana and Plantain (INIBAP) postharvest evaluation procedure. Out of these 2,040 plants, 40 somaclones were selected based on their better performance compared to the untreated plants (control). Results showed that prolonged subculture and addition of high concentration of 2,4-D produced both positive and negative variations. Positive variation was exhibited by heavier bunch weight, earlier flowering, longer shelf life and a larger number of hands, which translate into increased income. Negative variation, on the other hand, included dwarfism, delayed flowering and a lesser number of hands.

Key words: ‘Lakatan’ banana, postharvest traits, shelf life, somaclonal variation, 2,4-D

INTRODUCTION

In-vitro culture is a popular method of vegetatively propagating crop plants with high commercial value. Ideally, all plants generated from tissue culture are true-to-type i.e. have identical constitution from that of the mother or original plants. However, contrary to this, high incidence of off-types is a major concern. This variation is called somaclonal variation and may be defined as genetic and phenotypic variation among clonally propagated plants of a single donor clone (Kaeppler et al. 2000). Larkin & Scowcroft (1981) pointed out that a consistent proportion of the regenerated plants differ from the original parental type when submitted to tissue-culture techniques. Methods for detection of somaclonal variation have been explored for many years (Noval 1980). Although such variation may provide a useful source of genetic variability for crop improvement, it is undesirable in plant propagation. Moreover, Sultana et al. (2005) who worked on Indica Basmati rice, observed that tissue culture generates a wide range of variation ranging from 35.3% - 45.2%, which is related to the incubation time and is cultivar specific. Also, Smith (1998) observed that variation in plants derived from shoot tip culture can vary from 0-70% depending on the genotype. Therefore, this genetic instability can be a risk in germplasm handling and storage; however, it can also provide another source of novel and useful variability (Vuylsteke 1998) especially in crops where conventional breeding is difficult. Ehsanpour et al. (2007) observed that somaclonal variation was useful in selecting callus formation for desirable traits such as salt and drought tolerance or secondary metabolite production.

In Cavendish banana, somaclonal variation is associated with long duration in tissue culture and from a long period of the multiplication phase of meristem culture (Khayat et al. 2004). In another study, Skirvin et al. (1994) observed that somaclonal variation was related to growth regulator, cultivar variability, duration of in vitro culture, ploidy level and explant source. Furthermore, certain chemicals...
like 2,4-D also enhance somaclonal variation (Sultana et al. 2005).

According to Boranayaka et al. (2010), induced mutation is an important tool to create desired characteristics which are otherwise found in extensive germplasm collections. Moreover, variation resulting from micropropagation i.e. somaclonal variation, appears to be frequent in Musa such that somaclonal variation can be an effective tool in the improvement of horticultural traits of banana (Tang 2005). Larkin & Scowcroft (1981) suggested that somaclonal variation can be a useful source of novel variation for plant improvement. Similarly, Lestari (2006) reported that somaclonal variation can be an alternative technology in plant improvement which is expected to support conventional breeding. Through somaclonal variation desirable traits such as larger fruit size, more interesting flowers (in ornamentals), fruit texture and increased yield (Witjaksono 2003) may be obtained.

MATERIALS AND METHODS

Plant Materials
Corms of banana cv ‘Lakatan’ were obtained from a plantation in Magpet, North Cotabato, Philippines and were used for in-vitro propagation. Shoot tip cultures derived from ‘Lakatan’ corms were established using standard tissue culture procedures for disinfection, culture initiation and maintenance (Hwang & Ko 1990).

Shoot tips were inoculated on full strength Murashige & Skoog (MS) (1962) medium solidified with 7% agar supplemented with various levels of 2,4-D (3, 6, 9, 12 µM/L, respectively). Cultures were maintained at 27°C with 24 h white light provided by a 40 watt fluorescent lamp.

After 21 days, the initial cultures were further subdivided into 2-3 sections. Every 21 days thereafter, the cultures were transferred to a fresh medium. The cultures were maintained until the 15th subculture to determine the effect of age of culture on the induction of somaclonal variation in-vitro. Beginning on the third cycle until the 15th cycle, observations and data on the following was gathered: number of days from planting to flowering, number of days from flowering to harvest, number of days to 50% yellowing, number of days end of shelf life, bunch weight, average weight (fruit and hand), number of hands and fingers, fruit length, fruit girth, TSS (%Brix), pH, and percent dry matter content of fruit at harvest were recorded. Plantlets derived per treatment combination were planted in the greenhouse for four weeks.

The in-vitro derived plantlets were planted in a 2.5 hectare plot following a 3 x 3 m distance of planting and laid out in a Randomized Complete Block Design (RCBD) in factorial arrangement with 30 plants per somaclone (as replications). Field performance based on agronomic, pre-harvest (e.g. days from planting to flowering and days from flowering to harvesting) and postharvest parameters and shelf life were observed and recorded. Comparison observed through the performance of somaclones with different treatment combinations with that of the control or untreated sample plants using T-test and LSD was done.

Evaluation at the nursery level was also done by comparing the treated samples with the control. Candidate plants showing putative off-types were selected and used for further analysis.

RESULTS AND DISCUSSION

The effect of the various 2,4-D concentrations on the number of days to shoot formation, number of shoots formed previously established in MS culture media is shown in Table 1. Culture media supplemented with 9 µM 2,4-D were the earliest (49 days) to form shoots after initial subculture. In contrast, culture samples supplemented with 12 µM 2,4-D formed shoot 52 days after initial subculture. On the other hand, cultures supplemented with 3 and 6 µM 2,4-D (51 and 51.3 days, respectively) did not differ significantly with those without 2,4-D supplementations (50 days).

<table>
<thead>
<tr>
<th>Days to shoot formation</th>
<th>No. of shoots produced after 50 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 2,4-D</td>
<td>50.2±0</td>
</tr>
<tr>
<td>1 uM 2,4-D</td>
<td>51±0</td>
</tr>
<tr>
<td>6 uM 2,4-D</td>
<td>51.3±0</td>
</tr>
<tr>
<td>9 uM 2,4-D</td>
<td>49.7±0</td>
</tr>
<tr>
<td>12 uM 2,4-D</td>
<td>52±0</td>
</tr>
</tbody>
</table>

*CV 15.5%

In terms of number of shoots formed after 50 days, addition of 2,4-D significantly decreased the number of shoots produced. For example, shoot tips inoculated unto MS media without 2,4-D produced 32 shoots, while those shoot tips inoculated unto various 2,4-D concentrations (3, 6 and 12 µM 2,4-D, respectively) had significantly lower shoots produced (6, 7, 18, 5, respectively).

The above results implied that high 2,4-D concentration as a supplement to culture media had negative effects on the shoot initiation and multiplication of in-vitro culture bananas.
However, Iqbal et al. (2011) observed that 3 mg/L 2,4-D is very efficient in inducing embryonic callus with desirable quality friable callus in peanut. Likewise, El-Sayed et al. (2012) reported that 2,4-D and other chemicals can induce mutation in banana plants.

Multiplication rate of shoots from subculture 3 to subculture 15 in relation to the various 2,4-D concentration is shown in Figure 1. In general, a double sigmoid curve pattern of multiplication rate was observed. Initially, there was slow increase from subculture 3, to subculture 5 and a rapid increase from subculture 5 until subculture 9, then a rapid decrease from subculture 10 until subculture 11 and a sigmoid pattern from subculture 3 to subculture 9. All the other treatments followed the same trend. This is expected since multiplication rate in in-vitro culture follows an exponential increase, hence, this is a popular technique in mass producing vegetatively propagated plants e.g. banana (Akin-Idowu et al. 2009, Bhosale et al. 2011). Muhammad et al. (2004) reported that the maximum number of shoots was produced from each shoot tip after five subcultures.

Postharvest performance of 40 selected ‘Lakatan’ somaclones
The list of all potential somaclones and their corresponding beneficial traits is presented in Table 2. Criteria for selection were based on superior performances (both at harvest and postharvest) exhibited by the individual somaclones over that of the control. In terms of number of days from flowering to harvest, somaclone # 19 from cycle 10 treated with 12 µM 2,4-D (C10T5) was harvested 56 days after flowering. It means that it matured 32 days earlier than the control (88 days). This was followed by somaclone # 21 treated with 12 µM 2,4-D, subcultured until the 10th cycle which was harvested 65 days after flowering showing an earlier maturity of 23 days over the control.

Another important trait where significant variation was observed is in terms of bunch weight, where 19 somaclones were significantly heavier than the control. The heaviest bunch weight (20.78 kg) was observed from somaclone # 31 from cycle 11 without 2,4-D. This means a 7.78 kg heavier bunch than the control (12.10 kg), and 5.78 kg heavier than the usual normal Philippine ‘Lakatan’ which bears an average weight of 13.5 – 20.28 kg. These results indicate that prolonged subculture might be a factor that can induce the said beneficial or positive traits. It can be noted that somaclone # 20 derived from cycle 9 treated with 9 µM 2,4-D gave lower bunch weight, however, it yielded a higher number of fingers (20 fingers/hand) and the highest value of 32.2° Brix compared to the control which had an average of 16 fingers per hand and 26° Brix TSS. For the number of hands per bunch, seven somaclones were observed to have nine hands/bunch which was one hand/bunch more than the control (8 hands/bunch).

All of these seven somaclones were subjected to a prolonged subculture of 11, 12 and 13 cycles/subcultures but lower 2,4-D concentration. In terms of the number of fingers per hand, 29 somaclones gave one to five more fingers compared to the control which had 16 fingers per hand. These 29 somaclones had fingers ranging from 17-21 per hand. Furthermore, somaclone # 48 from cycle 14 treated with 12 µM 2,4-D took the longest (16 days) to 50% yellowing. This was nine days longer than the control which can be an indication of longer shelf life. Furthermore, 28 somaclones gave a range of 8-15 days

![Figure 1](image-url). Multiplication rate of banana shoot tips in response to various 2,4-D concentrations.
from green to 50% yellowing. Somaclone # 17 from cycle 12 treated with 9 µM 2,4-D (C12T4 # 17) and somaclone # 48 from cycle 14 treated with 12 µM 2,4-D had 20 days shelf life which was five days longer than the control (15 days). Another trait includes pH. It was observed that somaclone # 13 from cycle 13 treated with 9 µM 2,4-D/L showed a pH of 6.78 indicating that the fruit is not acidic. This might be interesting in later studies for nutrient/pharmaceutical consideration/properties.

Other somaclones had pH ranging from 4.43 to 5.57 while that of the control was 4.98. This might be an important indication of the nutritional quality of such somaclone. The results conformed to observation that tissue culture conditions can induce varied amount of genetic changes in different regenerated plants (Soniya et al. 2001). Overall observation showed that prolonged subculture and addition of high dose of 2,4-D can induce somaclonal variation. These findings conform to the results of Fukui (1983) that culture period itself is a mutagenic agent and that these mutations are not simply pre-existing in the ex-plant source. Lee & Philips (1987) and Zehr et al. (1987) stated that there is an “age effect” i.e. the longer the cultures are maintained, the higher the frequency of mutations found in the regenerated plants. Moreover, Mendez et al. (1996) reported that somaclonal variation can be due to the permanence of culture in-vitro or number of subcultures. Likewise, as previously stated, the presence of certain chemicals like 2,4-D also enhances the rate of somaclonal variation (Sultana et al. 2005).

Table 3 shows the summary of the selected somaclones and their performance. Somaclone # 31 from cycle 11 untreated with 2,4-D (C11T1 # 31) had heavier bunch (20.78 kg) than the control (12.10). In addition, somaclone # 5 from cycle 7 untreated with 2,4-D (C7T1 # 5), and somaclone # 42 from cycle 12 treated with 3 µM 2,4-D (C12T2 # 42) had heavier fingers (140 g) than the control (80 g). Somaclones # 5 and # 38 from cycle 11 treated with 3 µM 2,4-D had a larger number of fingers (21) per hand over the control (16).

The results of the study showed that indeed variation was induced by the treatments employed. Age (prolonged subculture) and high hormone level (2,4-D) were the factors that contributed to the positive and negative traits in somaclones. These results were in agreement with Roux (2004) indicating that the use of in-vitro mutagenesis and tissue culture have been found to make induction and selection of induced somatic mutations more effective.

Figure 2. Different somaclones of Banana cv ‘Lakatan’ with higher yield performance.
Table 2: Morphological traits of selected 'Lakatan' Somaclones from different plantations (4 replicates and 24 lines)

<table>
<thead>
<tr>
<th>Group</th>
<th>Plantation</th>
<th>Fruit (g)</th>
<th>Shelf life</th>
<th>Yellowing</th>
<th>Flowering to Harvest (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C11T2#9</td>
<td>16.80</td>
<td>11.87</td>
<td>110</td>
<td>140</td>
</tr>
<tr>
<td>2</td>
<td>C12T2#42</td>
<td>24.08</td>
<td>22.51</td>
<td>98</td>
<td>185</td>
</tr>
<tr>
<td>3</td>
<td>C13T2#13</td>
<td>21.60</td>
<td>20.46</td>
<td>120</td>
<td>160</td>
</tr>
<tr>
<td>4</td>
<td>C14T3#21</td>
<td>25.40</td>
<td>22.57</td>
<td>150</td>
<td>200</td>
</tr>
</tbody>
</table>

All data are significantly different from that of the control at 5% level (T-test)
Table 3. Summary of the selected somaclones and the corresponding traits exhibited expressed in response to prolonged subcultures and 2,4-D level.

<table>
<thead>
<tr>
<th>Somaclone</th>
<th>Heavier Bunch (kg)</th>
<th>Somaclone</th>
<th>Weight of finger (g)</th>
<th>Somaclone</th>
<th>Number of fingers/hand</th>
</tr>
</thead>
<tbody>
<tr>
<td>C11T1#31</td>
<td>20.78</td>
<td>C7T1#5</td>
<td>140</td>
<td>C11T2#15</td>
<td>21</td>
</tr>
<tr>
<td>C7T1#18</td>
<td>17.20</td>
<td>C12T2#42</td>
<td>140</td>
<td>C11T2#38</td>
<td>21</td>
</tr>
<tr>
<td>C7T1#27</td>
<td>17.00</td>
<td>C10T2#21</td>
<td>121</td>
<td>C7T3#5</td>
<td>20</td>
</tr>
<tr>
<td>C11T2#15</td>
<td>18.63</td>
<td>C11T1#31</td>
<td>120</td>
<td>C7T4#25</td>
<td>20</td>
</tr>
<tr>
<td>C13T2#1</td>
<td>19.15</td>
<td>C12T4#17</td>
<td>120</td>
<td>C11T2#9</td>
<td>20</td>
</tr>
<tr>
<td>C13T2#31</td>
<td>17.80</td>
<td>C14T3#21</td>
<td>120</td>
<td>C13T2#19</td>
<td>20</td>
</tr>
<tr>
<td>C13T2#19</td>
<td>17.00</td>
<td>C14T3#43</td>
<td>120</td>
<td>control</td>
<td>16</td>
</tr>
<tr>
<td>C14T3#21</td>
<td>17.00</td>
<td>control</td>
<td>80</td>
<td>control</td>
<td>80</td>
</tr>
</tbody>
</table>

leaf color), physiological variations (growth and sucker multiplication, duration of flowering, fruit ripening), and agronomic variations (bunch qualities) varied from 3 to 40% in the first generation, depending on the genotype. From a practical point of view, it is most important that off-types (whether positive or negative) can be identified at an early stage. For positive variation, farmers are assured of more benefits especially on yield translated into income while for negative variations, these can be culled out at the early stage to avoid loss in investments.

CONCLUSIONS AND RECOMMENDATION

With the foregoing obtained results, mutation induction through long culture period and high 2,4-D concentration may lead to enhanced heritable variation, hence, there is a need for further testing of advanced generations. This might be useful for functional genomics or breeding applications. This can be exploited for banana postharvest improvement. In addition, yield traits such as bunch weight can be further studied to develop new strains which will give better yield. Further testing of the selected mutant lines for the traits considered is also essential.

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LITERATURE CITED


