Genetic hybridization among genotypes of Taro (*Colocasia esculenta*) and recurrent selection for leaf blight resistance

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**Abstract**

Twenty six genotypes selected from the progeny of cycle-3 recurrent selection on the basis of having 10 suckers or more were screened for taro leaf blight (TLB) resistance using *in vitro* leaf-disc method which detected six genotypes with durable resistance. Field evaluation of the 26 genotypes, compared to the local control cultivar “Numkowec”, on the bases of TLB horizontal resistance, high comparable yield (dry matter, corm and cormel weight etc.) and good eating quality identified three superior genotypes, C3-10, C3-12 and C3-22. The three genotypes showed durable resistance to TLB and Coefficient of Infection (CI) = 20, 50 and 80 compared to CI=240 for the local control cultivar “Numkowec”. In a different study, the adaptability of the three genotypes were assessed in a range of agro-ecological locations and recommended for release. 

The breeding program was further advanced to generate cycle-4 population by performing 85 selective partial diallel crosses among the 26 genotypes. Crosses amongst the 26 genotypes were limited to those which flowered naturally or under the effect of gibberillic acid (GA). Various degrees of natural flowering occurred in 58% of the population; however, flowering was enhanced to 82% following the application of 0.5 gl\(^{-1}\) GA. Crosses among the remaining 23 genotypes were carried out by other workers following the same procedure. A total of 6000 vigorous seedlings generated from bulked progeny seeds of all crosses involving the 49 genotypes, were identified out of more than 10,000 seedlings, and transplanted to the field, constituting cycle-4 progeny population. A preliminary field evaluation of the population’s flowering ability, resistance to TLB and agro-morphological characteristics was carried out. 

A total of 237 genotypes with large size and non-deformed corms with no or little hair were initially chosen for further selection in a range of agro-ecological locations and release of elite lines, which will constitute the parental lines for cycle-5 recurrent selection population.

**Keywords:** *Colocasia esculenta*, recurrent selection, taro leaf blight

**Introduction**

Breeding taro for taro leaf blight (TLB) resistance in Papua New Guinea (PNG) was initiated in 1993 (Ivancic & Okpul, 1996). It is based on systematic improvement of populations through modified recurrent selection, and is divided into cycles. Each cycle is comprised of three steps including development of a new population, evaluation of genotypes in this population and recombination of superior genotypes to create a new population for the next recurrent cycle. The duration of each cycle varies from 12 to 18 months and the population size in each cycle generally exceeds 10,000 seedlings (Singh & Okpul, 2000). The base population for the current taro breeding programme consisted of 399 cultivated varieties from the PNG germplasm collection, 200 breeding lines from the Solomon Islands programme (majority of which were partly resistant to TLB) and wild genotypes of South East Asia and PNG origin (moderately or highly resistant to TLB) (Okpul, 1998). Cycle-1 population was developed by crossing resistant base population with superior local taro varieties. Cycle-1 was advanced to cycle-2 by intercrossing superior genotypes. Assessment of cycle-2 population for TLB resistance, yield and eating quality resulted in the selection of resistant genotypes with good yield and quality, which were intercrossed to generate cycle-3 population (Okpul et al., 1997). Under the existing taro breeding programme, for the third cycle of 49 genotypes were selected from the progeny population based on their resistance to TLB. Out of the 49 selected genotypes from cycle-3, 26 genotypes were advanced in this study to form part of the cycle-4 population through half diallel crossing. The resulting progeny was evaluated for agromorphological (above and underground) characteristics in addition to screening for resistance to TLB. The programme is focused on incorporating horizontal resistance in cultivars using systematic cyclic strategy (Singh & Okpul, 2000). The aim of the preliminary selection was to select the elite lines of cycle-4 for intercrossing to generate cycle-5 population. 

Taro has long been vegetatively propagated; therefore, domestication appears to have captured a limited proportion of genetic diversity of wild species, so
that majority of cultivars are probably clones from a narrow genetic base (Lebot et al., 2001). Due to this limited genetic variability, agronomic crop improvement in taro has been slow. Genetic recombination through hybridization among cultivars creates new genetic recombinants and can potentially improve quantitative traits like yield, eating quality and resistance to various pests and diseases (Ivancic & Okpul, 1997).

Hybridization of taro comprises of two steps, viz. emasculation and pollination, which determine the success of a breeding programme. Essential and desirable requirements should, therefore include good flowering ability, good seed set and high germination rate. Flowering was previously reported as quite rare among taro cultivars (Wilson, 1989; Ivancic & Okpul, 1997). However, with the relatively recent developments achieved in the artificial flowering induction methods (Alamu & McDavid, 1978), hand pollination methods (Strauss et al., 1980) and seed germination protocols (Strauss et al., 1979), the application of breeding methods for taro improvement can be highly successful (Wilson, 1984). This study aimed at intercrossing 26 elite lines of cycle-3 to generate cycle-4 progeny and identifying the superior genotypes for adaptability trials.

Materials and methods

Forty-nine genotypes, from cycle-3 progeny, selected on single plant basis for their resistance to TLB and superior agronomic traits were subdivided into two batches on the basis of availability of suckers (10 or more suckers), each comprising of 23 (Batch 1) and 26 (Batch 2) genotypes, respectively. Batch 2 was comprised of genotypes with 10 or more suckers. The plants were grown at the Wet-Lowlands Mainland Programme (WLMP) of the National Agricultural Research Institute (NARI), located at Bubia (10 miles outside Lae, PNG). The genotypes were planted in single rows such that five plants represented each genotype. The spacing was 0.5 m between plants and 1.0 m between rows.

This study oversees crossing individuals of Batch 2 (26 genotypes), whereas Batch 1 (23 genotypes) was managed by the taro breeding programme staff. Separate crosses were conducted on the two batches. However, resultant seeds from the crosses were later bulked and germinated in the greenhouse.

Induction of flowering

Gibberellic acid (GA) was applied on the plants, two months after planting, at a concentration of 0.5g/l as described by Ivancic (1992). The GA spray solution was prepared shortly before use, and spraying on the plants was done early in the morning, using a hand-pump sprayer. All leaves were sprayed lightly on both sides while a little GA solution was sprayed into the cup formed by the petiole bases. Spraying was carried out on a day when it was not expected to rain, to avoid washing of the GA. Genotypes were grouped, based on flowering rate per plant, into four categories. Genotypes, producing no flowers, were placed in the non-flowering category (0) whereas those producing 1-2 flowers, were included in the Low flowering category (1) and the ones bringing forth 3-4 flowers were grouped in the moderately flowering category (2) and lastly those yielding 5 flowers or more constituted the vigorously flowering category (3).

Hybridization

Crossing design: Half diallel design without reciprocal crossing and selfing was used in crossing the 26 selected genotypes of cycle-3 progeny which produced flowers. Crossing was conducted from December 1999 to April 2000. If all the 26 parents flowered, a total of 325 crosses in a half diallel would have been possible as per the following formula: \[\frac{(n-1)^2 + (n-1)}{2}\], where \(n\) is the number of parents used in crossings. However, the only 21 parents that flowered were used in the crosses.

Emasculaton and hand-pollination: Emasculation and pollination were carried out together on the inflorescence of female-parent plant before pollen was shed as described by Wilson (1989) and Ivancic (1992).

Hand-pollination was carried out as described by Wilson (1989). On the female-parent plant, an inflorescence that was at the ‘crack-stage’ distinguished by strong aroma was chosen and the bag covering it was gently removed. The male portion of the spadix was cut-off by cutting through the band of sterile flowers that separated the male flowers from the female flowers without removing the spathe. Afterward, the spathe surrounding the female portion was carefully cut away, as not to damage the peduncle. Simultaneously, on the male-parent inflorescence, the male portion was cut-off from the spadix, held by the sterile tip and was carefully removed from the spathe. On the male-parent plant, a bagged inflorescence with pollens was identified. The entire inflorescence was cut-off and taken to the female-parent plant. The inflorescence was gently carried to avoid shaking-off pollens.

Pollination was performed before midday, preferably early in the morning, by applying pollen on the stigmas with a small brush. Pollens were evenly applied to cover all the fertile female flowers to make sure no fruithead with a few berries would develop. After completing pollination, the spathe of the female inflorescence was carefully replaced. The pollinated inflorescence was further protected by covering with a brown paper bag. This was to avoid contamination from foreign pollens as well as to protect from any likely rain from washing-off the pollens being applied. Three days after pollination, pollinated inflorescence were unbaged.

To label pollinated inflorescences, metal tags were tied, with tire wires, around the peduncle of inflorescences. Labels carried information related to parentage (the name of the female-parent plant was
recorded first followed by the male-parent plant) of the cross, the date of pollination and initials of the pollinator.

Seed development generally took 28-35 days after pollination. To harvest seeds, each hand-pollinated fruithead was picked with its label attached and taken to a water source for extraction of seeds. Extracted seeds were then placed on a piece of paper and left to dry on a bench without being exposed to direct sunlight. Dried seeds were kept in labeled paper bags until sowing.

Seed sowing

Sowing seeds from harvested crosses was initiated in May 2000. Approximately 100-150 seeds were sown in each 25-cm-diameter plastic pot filled with adequate soil (just below the rim of pot) and placed into special waterbeds in the greenhouse. Each waterbed was prepared by placing a transparent plastic sheet over four lumber off-cuts placed to form a rectangular block. The lengths of the lumbers were dependent on the number of pots and available space in the greenhouse. Water was collected on the plastic sheet upon which the pots were finally placed. A separate sheet was placed over the pots to retain moisture. Seeds germinated approximately 5-7 days after sowing. Three weeks after germination, seedlings were thinned to a maximum of five per pot.

Management of seedlings

A population of more than 10,000 seedlings was raised in the greenhouse. However, based on plant size, only 8000 were transferred to the shade nursery in September 2000, when they had reached the 3-5 leaves growth stage and this was approximately 4 months after germination. The shade nursery was used to harden-off plants and identify vigorous seedlings.

Out of the 8000 in the shade nursery, 6000 vigorous ones were identified and transplanted from the shade nursery to the field in January 2001.

Seedlings were planted in two batches from which genotypes obtained were tested for resistance to TLB and their agro-morpho characters determined. Evaluation of yield and eating quality was later carried out when the population size was further reduced.

**Evaluation of cycle-4 progeny population**

Field assessment of agro-morphological (aboveground) characteristics: Preliminary evaluations were made on several aboveground plant characteristics of importance for selection of genotypes. Characteristics included sucker ability and flower production. These characteristics were observed at six months prior to harvesting. Only were non-flowering genotypes having a minimum of three suckers selected. Genotypes producing flowers were discarded, as this was an indication of association with wild characteristic(s).

**Screening for resistance to taro leaf blight:** Screening for resistance to TLB was carried out using the spraying technique as described by Singh & Okpul (2000). Six months after planting out in the field, plants were inoculated with a spore suspension prepared from local isolate of Phytophthora colocasiae prevalent at Bubia. For the preparation of the spore suspension, approximately fifty leaves with young TLB lesions were placed in a bucket containing moistened newspapers for maintaining high humidity. Leaves were incubated overnight in a dark room at 22-24°C, after which approximately one kilogram of leaves was squeezed in three litres of water. The resultant spore suspension was filtered prior being used for inoculations. The entire plant was sprayed and observed for response to TLB. Following observations based on percentage leaf area

### Table 1. Ranking of corm characteristics used in selection of corms

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Ranking</th>
<th>Description of corm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>1</td>
<td>Large (&gt; 500 g)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Medium (&gt;200 - 500 g)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Small (&gt;100 - 200 g)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Very small (100 g or less)</td>
</tr>
<tr>
<td>Shape</td>
<td>1</td>
<td>Very good (non-deformed; with no cormels)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Good (slightly deformed with few cormels)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Fair (deformed with some cormels)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Bad (completely deformed)</td>
</tr>
<tr>
<td>Hairiness</td>
<td>1</td>
<td>No hair cover (0 %)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Little hair cover (50 %)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Some hair cover (75 %)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Hairy 100 %)</td>
</tr>
</tbody>
</table>

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**Fig. 1. Number of crosses made on flowering genotypes as determined by flowering intensity**

Research article

“Genomic selection of taro for leaf blight resistance”

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damage, genotypes displaying moderately resistance reactions were selected for further evaluation while those showing highly susceptible and hypersensitive reactions were discarded, as per adopted selection strategy for polygenic/horizontal resistance breeding.

Field assessment of underground (corm) characteristics:
The underground characteristics observed for selection was corm size, shape and hairiness. Observations on these characteristics were done at approximately seven months during harvest. These observations were based on ranking described and outlined in Table 1. The corms with ranking 1 or 2 for all three characters (size, shape and hairiness) were selected for further evaluation.

Results and discussion

Flower production in parental population
The tested 26 genotypes varied with respect to their flowering ability such that natural flowering was absent in 42% of the genotypes but, occurred to various degrees in the remaining 58% (Table 2). When flowering was induced by GA application, flower induction failed with 19% of the population, but, succeeded, though with various degrees with the remaining 82%. Genotypes were grouped according to flowering intensity into four categories as outlined in the materials and methods section. Analysis to compare natural with GA-induced flowering showed that there was no significant difference (P<0.05) between the two. There was also no significant difference between the four flowering categories under natural and GA induced flowering regimes.

The occurrence and synchronisation of flowering in taro are important criteria in selecting parents for crosses, hence the success of breeding programmes. Natural flowering frequency in the majority of wild taro populations is very high under optimal environmental conditions (Ivancic, 1995). However, natural flowering in cultivated taro is rare (Plucknett et al., 1970; Purseglove, 1972; Hay, 1990). The non-uniform flowering in taro is one of the major factors limiting classical intraspecific hybridization of the crop (Ivancic, 1995). Nonetheless, with the high frequency of flowering in wild taro populations, improvement of flowering in taro can be achieved with the incorporation of wild genotypes into breeding programmes (Ivancic et al., 1996).

Several methods are available for synchronisation of flowering in taro. The methods include spraying the parental material with gibberellic acid (GA), replanting after one flowering cycle is over, inducing stress by drought or varying temperatures and removal of cormels, suckers and stolons. However, spraying the parental material with GA was considered the most efficient and reliable method (Ivancic 1992). Induction and synchronisation of flowering using gibberellic acid (GA) was reported in C. esculenta and Xanthosoma sagittifolium (McDavid & Alamu, 1976; Cable, 1979; Wilson, 1981). Various GA concentrations and methods of application have been reported by Wilson (1981). By contrast, GA application in this study used the recommended dose of 0.5 g/l (Ivancic, 1992), which increased flowering from 58% to 82% in the selected genotypes of cycle-3. Twenty-one genotypes (82%) of the parental material of 26 genotypes produced flowers as compared to only 15 genotypes (58%) under natural flowering. On the other hand, the number of non-flowering genotypes was also reduced from 11 genotypes under natural flowering to only 5 genotypes with GA application. However, it was previously reported that some taro varieties did not often respond to GA application (Ivancic et al., 1996). Further more, natural flowering was also said to be partially genotypic-specific (Ivancic & Lebot, 1998; Goenaga & Hepperly, 1990). Taking such reports into consideration, it is

<table>
<thead>
<tr>
<th>Flowering Group</th>
<th>Genotypes</th>
<th>No. of genotypes</th>
<th>Frequency (%)</th>
<th>Flowering occurrence</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural flowering</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-flowering</td>
<td>C3-3, C3-6, C3-10, C3-27, C3-30, C3-35, C3-36, C3-41, C3-42, C3-43, C3-48.</td>
<td>11</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flowering</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>C3-22, C3-40.</td>
<td>2</td>
<td>8</td>
<td>1-2</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>C3-12, C3-26.</td>
<td>2</td>
<td>8</td>
<td>3-4</td>
<td>2</td>
</tr>
<tr>
<td>Vigorous</td>
<td>C3-14, C3-16, C3-17, C3-18, C3-19, C3-28, C3-29, C3-32, C3-33, C3-34, C3-45.</td>
<td>11</td>
<td>42</td>
<td>&gt;5</td>
<td>3</td>
</tr>
<tr>
<td>GA induced flowering</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-flowering</td>
<td>C3-6, C3-10, C3-35, C3-36, C3-41.</td>
<td>5</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flowering</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>C3-3, C3-43, C3-48.</td>
<td>3</td>
<td>12</td>
<td>1-2</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>C3-22, C3-30, C3-42</td>
<td>3</td>
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<td>2</td>
</tr>
<tr>
<td>Vigorous</td>
<td>C3-12, C3-14, C3-16, C3-17, C3-18, C3-19, C3-28, C3-29, C3-32, C3-33, C3-36, C3-27, C3-34, C3-40, C3-45.</td>
<td>15</td>
<td>58</td>
<td>&gt;5</td>
<td>3</td>
</tr>
</tbody>
</table>
tempting to suggest that these factors could have been contributing to the non-flowering behaviour observed in minority of selected genotypes of the cycle-3 parental material. Additionally, genotypes with small size were also observed to be flowerless.

Although the incorporation of wild genotypes into the breeding programme succeeded in promoting flowering in the progeny, it was found to cause the development of undesirable wild traits like stolon formation and acridity of Corms which were entrenched into the progeny. It was therefore decided to focus on removal of undesirable traits, such as dense flowering, associated with reduced size (negative selection) prior to selecting of desirable genotypes (positive selection).

Hybridization

The selective crosses done on the 21 flower-producing genotypes of the 26 parental materials resulted in a total of 85 half diallel crosses. Flowering intensity determined the number of crosses made to each of the genotypes. The number of crosses per genotype ranged from one to twelve (Fig.1).

A total of 80 additional crosses resulted from crossing the other 23 cycle-3 parental genotypes of Batch 1. The resultant seeds from these crosses were bulked with those derived from the crossing of the 26 genotypes of Batch 2 to attain maximum genetic recombination of cycle-4 in order to keep the genetic base of the next cycle broad. Bulked seeds were germinated in the greenhouse, generating > 10,000 seedlings (cycle-4 progeny).

While half diallel crossing had widely been implemented in breeding many crops, it was recently adopted in breeding of taro. Half diallel without reciprocal crosses used in crossing the selected genotypes, was considered adequate in the absence of maternal effect. Fyfe & Gilbert (1963) cited in Clerge le (1966) had previously discussed the advantages of half diallel over other forms of the diallel design. One of its greatest advantages is, it gives maximum information about the architecture of a trait, parents and allelic frequency (Kearsy, 1965 cited in Singh & Singh, 1983).

In this study, incomplete half diallel was used for crosses since a certain proportion of plants in the crossing nursery were small in size and non-flowering. As a consequence, the expected number of crosses for the half diallel design was not fully feasible. However, this constraint was not considered serious since the number of seedlings (10,000) that resulted from the number of crosses performed was an adequate representative of the cyclic progeny. Considering resources (time and manpower) available for WLMP, it was almost impractical to handle a progeny of more than 10,000 seedlings efficiently if the expected crosses were to be made. It may be argued that incomplete diallel may lead to narrowing down the genetic base because of loss of allelic frequency from the gene pool of the base population. A base population of 20 parents is considered sufficient to achieve maximum genetic gains and recombination using recurrent breeding approach (Robinson, 1996). However, in the present cycle, a second batch of 23 parents were also crossed in a half diallel design in addition to 85 crosses made in the current studies. So overall, a total of 49 parents (23 genotypes from Batch 1 and 26 genotypes from Batch 2) were used to keep the genetic base of next cycle broad and to avoid any inbreeding depression that is likely to occur as a consequence of untried crosses.

Evaluation of cycle-4 progeny

A preliminary selection aimed at reducing the population to a manageable size was carried out on the genotypes in the field which were cycle-4 progeny population. Using the selection criteria outlined in Table 3, the size of the population was reduced during each stage of selection. Selection at six months based on flower production and sucker ability resulted in the selection of 30% of the population (Table 3). The genotypes selected were those which did not produce flowers and had a minimum of three suckers. Screening for resistance to TLB carried out on the selected 1800 genotypes had resulted in selection of 40% of the population (720 genotypes). Genotypes selected were those showing moderately/horizontal disease reactions as per selection strategy for polygenic/horizontal resistance breeding. Highly resistant or susceptible genotypes were eliminated. The final selection done on corm characteristics during harvest resulted in the selection of 237 superior genotypes for further evaluation at various locations throughout PNG for wider adaptability and selection of parents for recombination to generate the next breeding cycle (cycle-5). With the advancement of breeding cycles, the programme is in the process of identifying more superior genotypes. Upon completion of advanced trials for cycle-4, new varieties resistant to TLB,
which are high yielding with good eating quality will be released and distributed to farmers.

References