HYBRIDIZATION OF TARO (*COLOCASIA ESCULENTA* (L.) SCHOTT.): FLORAL DEVELOPMENT AND STIGMA RECEPTIVITY.

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ABSTRACT

The floral development and the duration of stigma receptivity in Colocasia esculenta *was studied at the Bubia Agricultural Research centre, near Lae in the lowlands of Papua New Guinea. In determining the duration of stigma receptivity, female components were pollinated at different stages of their development. Although taro is a protogynous species, results showed that there was overlapping between the male and female developmental stages of the inflorescence. The period of stigma receptivity lasted 10 days. The stigmas were optimally receptive between 2 to 8 days after the emergence of the inflorescence. The effects of stressful climatic conditions on the flowering process were also observed. Drought stress may affect both natural and artificial hybridization.*

Key words: Taro, Colocasia esculenta, flowering, seed setting, Morobe Province.

INTRODUCTION

Taro, *Colocasia esculenta* (L.) Schott., is an edible aroid belonging to the monocotyledonous family, Araceae. It is an important crop in the Asia-Pacific region because of its cultural, economical and dietary values. The status of the crop within the region, together with its declining trend in production (Jackson and Gollifer 1975; Bourke 1982), has prompted breeding work to improve production. Population improvement of taro, based on a modified recurrent selection method, was pursued more recently by Ivancic (1992) to create more productive genotypes, resistant to main disease and to improve production.

Good flowering ability, good seed set and high germination rate are the most important characteristics in conducting breeding work but flowering is relatively rare among taro cultivars. However, improvement of these traits through the involvement of wild and semi-wild genotypes (with outstanding flowering ability) in a recurrent selection programme was achieved by Ivancic *et al.* (1994). Flower-inducing hormones such as gibberellic acid can also be effectively used to improve flowering. Further, seed set and germination rate depend on pollination success and pre- and post-zygotic developments respectively. Hence, a better understanding of the floral biology and phenology is necessary to enhance breeding work.

This study aimed at investigating and determining the duration of stigma receptivity in order to facilitate intra-specific hybridization. Observations on floral development and the effects of stressful climatic conditions on flowering and seed setting are also discussed.

Materials and Methods

The study was conducted from November 1994 to April 1995 at Bubia Agricultural Research Centre (BARC), near Lae, Papua New Guinea (PNG). The centre is situated at 146°4′E, 6°41′S at an altitude of 20 m a.s.1. and receives a mean annual rainfall of 2870 mm.

All genotypes used in these studies were part of the existing breeding programme. They included hybrids originating from the Solomon Islands and PNG (developed from the breeding work) and wild genotypes. The genetic improvement programme and the genotypes used have been described elsewhere (Ivancic *et al.* 1994).

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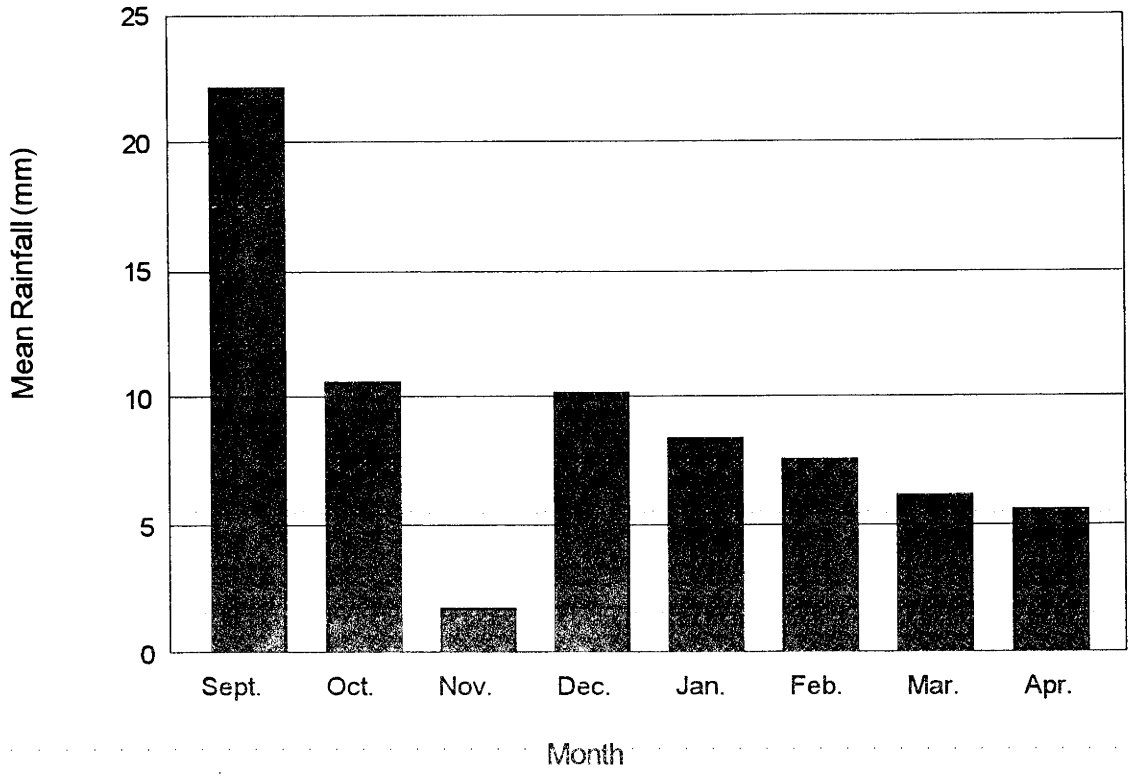
Figure 1. *Mean Monthly Rainfall: September 1994 - April 1995*



Floral development was studied by closely monitoring inflorescences in different genotypes.

In order to determine the duration of stigma receptivity, female components were pollinated at different stages of their development. The stage at which the spathe emitted the insect-attracting fragrance was denoted as 'day zero', or the beginning of anthesis. Inflorescences were emasculated and artificially pollinated daily starting 6 days before 'day zero' (a day before the inflorescence emerged out of the petiole sheath) and up to 5 days after anthesis had begun. Female components planned for pollination after 'day zero' were isolated when the spathe was still green and closed. Isolation was achieved using hard paper envelops (13 x 20 cm) closed with paper clips. Emasculation was done on the 'day zero'. The isolated pistillate flowers were then artificially pollinated later on selected days.

After pollination, female flowers were further protected to avoid contamination from foreign pollen by wrapping them with a thin layer of cotton wool and the lower part of the spathe. Parentage and age of the fruit head was indicated on a label tagged around the inflorescence's peduncle.

The mature fruit was washed in water and sieved using a strainer (0.4 mm pores), and the seeds were then dried at 18°C in an air-conditioned room. The air-dry weight was determined after 5 days. The number of seeds was estimated using the 1000-seed weight of 0.184 g (mean obtained from 0.198 g (Ivancic *et al.* 1994) and 0.170 g (this work)).

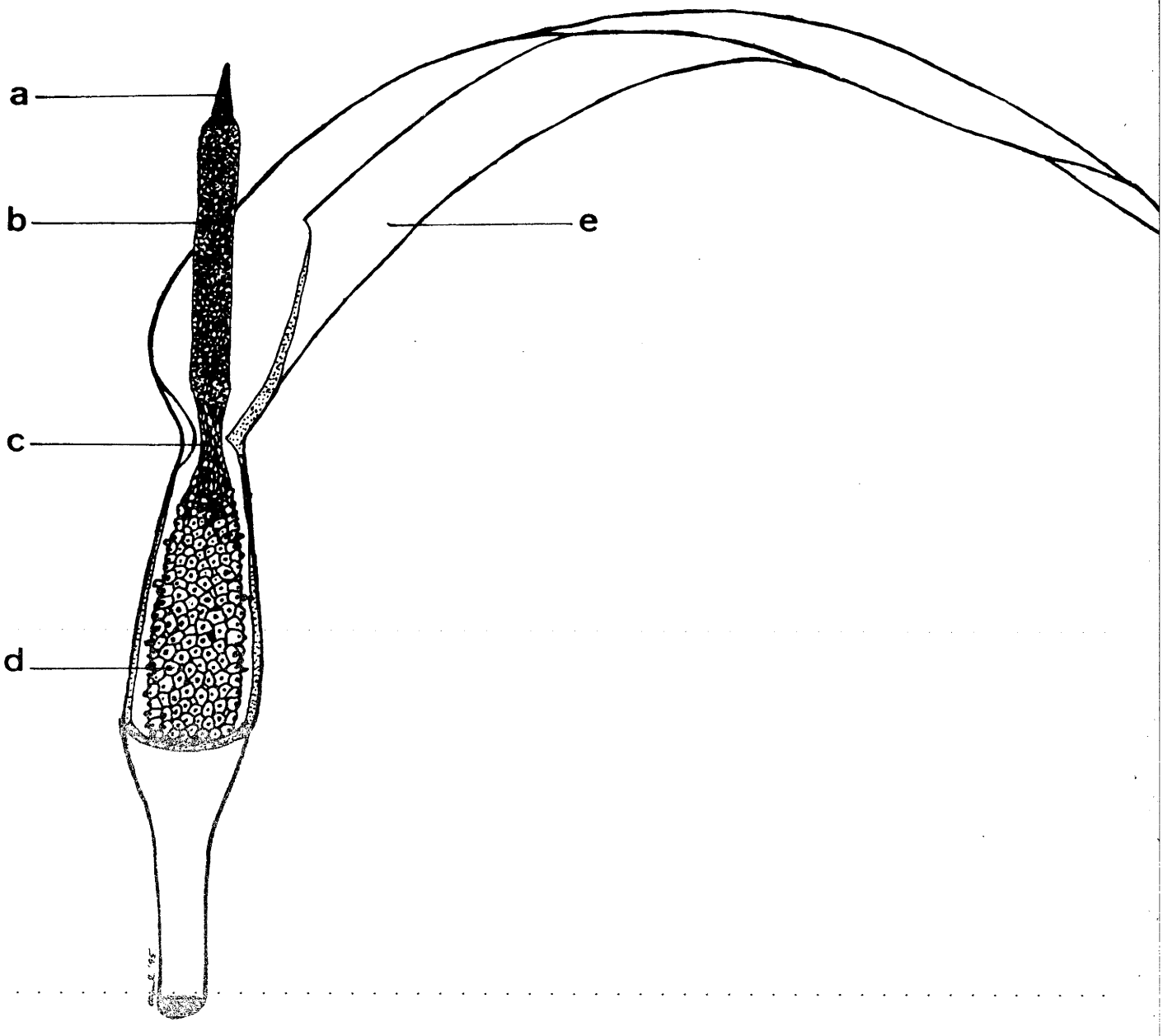
The effects of stressful climatic conditions were determined mainly by observations of the breeding plots and wild populations. The effect of the dry spell experienced between October and December 1994 (Figure 1) was evident in the December 1994 to January 1995 period. The effect of the wet weather, especially rainy days, was observed between December 1994 and April 1995.

RESULTS

Floral Development

Flowering in *C. esculenta* under Bubia climatic conditions is not seasonal. Genotypes with good

Figure 2. *Inflorescence of taro (with spadix exposed): (a) sterile appendage, (b) male portion, (c) sterile band, (d) female portion, and (e) spathe*



flowering ability flower throughout the year.

C. esculenta has fragrant monecious inflorescence which exhibits protogyny (Figure 2). More elaborate descriptions of taro inflorescences are given in other literatures (Shaw, 1975; Strauss, 1983; Purseglove, 1988). A plant of a cultivar generally develops up to three floral clusters during its growth period, an average of six months. Wild genotypes can develop more. Each floral cluster may develop one to five inflorescences. Shaw (1975) reported similar observations.The number of floral clusters and inflorescences per cluster vary between genotypes.

The development of each inflorescence, from its emergence to when the fruit head fully ripens, takes approximately 35 days. The appearance of the flag leaf signals the emerging inflorescence (Ivancic

Table 1. *Mean seed dry weight and estimated number of seeds obtained at various stages.*

Floral Development Stage *	No. of Crosses	Seed Dry Weight (g)		Number of seeds	
		Mean	Range	Mean	Range
-6	5	0	0	0	0
-5	8	0.12	0.04 - 0.54	625	217 - 2935
-4	8	0.37	0.04 - 1.61	2004	217 - 8750
-3	6	0.73	0.31 - 1.82	3967	1685 - 9891
-2	4	0.80	0.20 - 1.71	4335	1087 - 9294
-1	5	0.81	0.63 - 1.43	4394	3424 - 7772
0	5	1.21	0.62 - 1.82	6576	3370 - 9891
1	6	0.74	0.11 - 1.81	4004	598 - 9837
2	6	1.03	0.33 - 1.58	3768	1794 - 8587
3	7	0.51	0.67 - 1.13	2772	3641 - 6141
4	7	0.07	0.09 - 0.24	373	489 - 1304
5	5	0	0	0	0

* *Day relative to age when the fragrance is emitted (i.e. 'day zero').*

1992). The flag leaf usually develop together with the new leaf, partly wrapping it. It indicates the differentiation of a floral cluster, and may appear as early as 19 days before the emergence of the first inflorescence. At emergence, the inflorescence may generally be green, purple or have various distributions of colours depending on the general pigmentation of the genotype. Five days after emergence, the spathe generally turns yellow, unrolls and slightly opens. This happens with simultaneous emission of the insect-attracting fragrance. This observation is based on the first inflorescence. Inflorescences that develop later may be exposed out of the petiole sheath early (taken as emergence) and thus it may take longer (up to eight days) before they unroll and release the fragrance. Anther dehiscence begins on the following morning and continues up to 24 hours. Prior to anther dehiscence, the neck area and the lower portion of the spathe closes in to isolate the pistillate flowers. The upper limb of the spathe, together with the male component of the spadix, then withers and eventually dries off after two days. Following successful fertilization and seed development, the fruit ripens as early as 21 days later. At this stage the portion of the spathe covering the fruit head splits longitudinally around the middle or slightly below, and rolls back to expose the ripe berries. The peduncle becomes weak and eventually falls with the fruit head.

Interestingly, seed germination was observed at the base of the mother plant in the breeding field at BARC. This was observed during the wet season. The authors suspect abiotic factors such as shade (a result of competition), moisture and nutrient unavailability as probable causes of the inability of taro to propagate naturally through seeds.

Stigma Receptivity

Detailed description of the morphology of pistillate flowers were given by Shaw (1975), Strauss (1983) and Purseglove (1988), but, stigma receptivity was not reported. Strauss *et al.* (1980), however, noted that stigmas became receptive on the day when pollen shedding began and remained so for two to three days thereafter. The present study found that the stigmas gradually became receptive over time, beginning at the day when the inflorescence emerged from the petiole sheath.

Seeds were obtained from the stage of floral emergence i.e. day - 5 (mean of 625 seeds) to 9 days later on day 4 (mean of 373 seeds), peaking (mean of 6576 seeds) on day 0 when the fragrance was released from the spathe (Table 1).

Genotypes vary in their potential to set seeds. We observed that some can produce over 10,000 seeds per fruit head. The overlapping ranges in dry seed weight and number of seeds (Table 1) may also indicate variation in number of fertile flowers, number

Table 2. *Effect of drought on seed setting*

Insect pollinated inflorescence (%)			Artificially pollinated inflorescence (%)	
with mature seeds	with immature seeds	with no seed formed	with mature seeds	with immature seeds
2 (3/162)	14 (22/162)	84 (136/162)	79 (140/177)	21 (37/177)

of parietal placentae and ovules per ovary, and seed size and weight between genotypes as earlier reported by Shaw (1975). As a logical result, there are differences in their seed production potentials.

Climatic effect on flowering

The development of inflorescences within floral clusters was noted to be accelerated during the dry period. This was manifested in early maturation of inflorescences inside the petiole sheath, rapid production of consecutive inflorescences, inhibition of anther dehiscence, lack of or only partial emission of the insect-attracting fragrance, and wilting of immature fruit heads. Reduction in the complete spadix was also noted.

Insects, especially Drosophillidae, flies and bees, which may be pollinators, were found to be present. Despite these, seed setting of only 16% was recorded following the November 1994 drought in natural crosses (Table 2). This low percentage of seed set may be attributed to scarcity of pollen donors; decrease or lack of fragrance to attract pollinators; and in some cases, the development and receptivity of inflorescences inside the petiole sheath which prevents or impedes insect (pollinators) entry. Seed setting was better in artificial crosses (100%). The effect of water stress resulting in wilting of immature fruit heads has been observed in both artificial (21%) and natural crosses (14%).

On the other hand, wet weather, especially rains, affected pollen production and availability. It was noted that either anther dehiscence was inhibited or the pollen became too wet to interest insect visitors. However, inflorescences in anthesis after rains were noted to have full pollen production.

DISCUSSION

C. esculenta flowers throughout the year under the Bubia climate. This may be attributed to constancy of environmental conditions (such as daylength) or the crop's insensitivity to environmental fluctuations (Frankel and Galun 1975). Depending on its flowering ability and growth period, one plant generally produces up to three floral clusters. Its fragrant monecious inflorescences exhibit protogyny. Impediment of self-pollination (in the 'strict sense,' i.e. transfer of pollen from the male portion to the pistillate flowers on the same inflorescence) is effected by the morphological development of the spathe, which at least partially isolates female flowers in space. In some genotypes, the neck portion of the spathe is not efficiently closed and so self-fertilization may result from pollen carried down the spadix by gravity, rain water and insects. Interestingly, less than 2% successful self-pollination ('in the strict sense') was observed from inflorescences isolated and exposed to rain after pollen has been shed. However, such selfing is rarer than geitonogamous pollination (pollination between clones of one genotype). Cross-pollination (including geitonogamy) is done mainly by insects and occurs at the stage when the spathe unrolls with simultaneous release of the insect-attracting fragrance. Nevertheless, there is an overlap between the male and female developmental stages of the inflorescence. The stigmas of the female flowers were receptive from the stage of their emergence until the fourth day after anther

dehiscence had begun.

From the result, it seems that most, if not all, stigmas of the female flowers are fully receptive when the spathe emits the insect-attracting fragrance. It should be noted that this study did not measure the duration of stigma receptivity in individual female flowers. Thus, the progressive increase and decline in seed set (Table 1) could possibly reflect the number of receptive stigmas out of the total number of female flowers.The lack of seed set may be attributed to factors affecting pre- and post-zygotic development and other problems associated with pollen dispersal. However, lack of seed set and low seed numbers in this study could be the result of either immature stigmas or loss of their receptivity due to senescence.

In breeding, synchronizing the flowering to cross desirable individuals can be a serious problem. This is especially obvious in cases where pollen production is hindered or when only a limited number of inflorescences, which can be used as females, are available. As such, the 10-day long period of stigma receptivity can lessen or alienate such concern, and also enable flexibility of hybridization in breeding work. To achieve good seed set, it would be wiser to pollinate the female flowers at the stage when they are optimally receptive, which is between 2 to 8 days after the emergence of the inflorescence.

In the natural system, earliest pollination takes place when the spathe starts to unfold and releases the insect-attracting fragrance. Female and male portions are early isolated on the spadix, and later, the female flowers are isolated by the constriction in the spathe. Earlier pollination has to be conducted artificially, although some abnormal inflorescences with exposed pistillate flowers may receive pollen by wind. Seed set in nature for entomophilous plants is strongly dependent on the presence of insect pollinators, prevalence of inflorescences in their male phase and availability of pollen within or between populations, the distances between flowering plants, and the probability that insect pollinators will visit these inflorescences (Young 1990).

Drought and wet weather may affect the flowering process and seed development. Acceleration of floral development due to drought stress has been observed in many cultivated plants (Blum 1988). Drought stress is of especial concern in taro as it hinders pollen production and dispersal and seed development in both natural and artificial crosses. Stigma receptivity and pre-zygotic development may have been very little or not affected. This was obvious from the better seed setting in artificial crosses. Immature seeds obtained in artificial crosses were mainly caused by wilting of the peduncle and fruit head which affected seed development. This may be attributed to drought-induced water stress because viable seeds were usually obtained when the inflorescences are cut at the peduncle and placed in water. In counteracting stress induced by drought, the PNG breeders usually use irrigation systems, especially flood and sprinkler irrigation. On the other hand, in the wet period only the paternal parent was affected: anther dehiscence was inhibited or pollen became too wet to interest insect visitors. Therefore, to ensure that pollen is shed, inflorescences (in the stage when the spathe unfolds and emits the fragrance) should be cut at the peduncle and placed in water in an air-conditioned room. Pollen will be available on the following morning.

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