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Floral biology and its manipulation for successful breeding programs in cassava (*Manihot esculenta* Crantz) - A review

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Abstract

Cassava is an important staple food of the tropical regions, and it fulfils the dietary requirements of ~900 million people. It is a strategic crop with climate resilience, wide adaptation to diverse climatic conditions and has the potential to cope with the effects of climate change in the future. Cassava is a cross-pollinated crop, pollinated mainly by honey bees. Speed breeding incassava is constrained by its high levels of genetic heterozygosity, sparsely flowering nature of genotypes with high breeding value and low seed set as well as seed germination. In India, cassava breeding was successfully carried out during the last five decades through selection of profusely flowering genotypes, manipulation of flowering in sparsely flowering genotypes using mechanical methods and hormonal treatment. There is variation in flowering time (from 4 months to >eight months) and the number of flowers among genotypes. Different genotype-environment interaction existed on flowering among varieties and a strong environmental effect on the number of flowering peaks within the same variety was noticed. Induction of flowering in erratic flowering types was done through pruning/de-topping of non-branching types and by grafting on to profusely flowering clones. Cryopreservation of pollen of sparsely flowering genotypes was successfully undertaken to maximise the genetic recombination in cassava. The present paper summarises the floral behaviour of cassava in different mating systems and artificial induction of flowering and cryopreservation of pollen that was successfully used to develop hybrids, inbreds and triploids in cassava.

Keywords: Cassava, flowering, Growth regulators, Grafting, Polyploidy, Cryopreservation

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important starch-yielding crops grown in many parts of Asia, Africa and South America. It is grown throughout the tropics for its tuberous roots, from which cassava flour, starch, sago, biofuel, bioplastic, biopesticides, etc. are produced. Cassava is the sixth most important source of calories in the human diet. This crop is an essential carbohydrate source for humans and animals, having higher energy production than other root crops, 610 kJ 100 g^{-1} fresh weight (FAO, 2017). Cassava is cultivated in about 224 countries on 26.64 million hectares worldwide, with an annual production of about 296.86 million tons (FAO, 2017). In India, it is cultivated in 1,99,000 ha with a yearly production of 41,71,000 tonnes.

Cassava is a native to South America and the Southern and Western Mexico. It is one of the first crops to be domesticated, and there is evidence that it was grown in

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Peru about 4000 years ago and in Mexico about 2000 years ago (Duke et al., 1983). It has been generally believed that cassava originated from Brazil and Paraguay (Olsen and Schaal, 1999). It spread throughout Central and tropical South America and was taken by the Portuguese to Africa in the 16th century. Portuguese travellers from Brazil introduced cassava to India in northern Kerala during the 17th century. Later, cassava became important as food, feed and for industrial use in India and other Asian countries.

Cassava is grown throughout the tropical regions between latitudes 30° N and 30° S and is potentially one of the most efficient carbohydrate producing crops. It is adapted to temperatures ranging from 18°C to 25°C, rainfall of 50 to 5000 mm annually, and poor soils with pH ranging from 4 to 9. It is an erect growing shrub, branched or un-branched, with palmate partite leaves. The improved varieties were mainly developed through hybridization, followed by clonal selection.

In this review, we describe the floral biology of cassava and its variation in different mating systems. Various approaches adopted to manipulate the flowering for accelerating cassava breeding programs are discussed in detail. The paper also focuses on strategies that involve regulating floral transitions through hormonal application, gene expression and cryopreservation of pollen for long-term use.

Floral biology

Cassava is monoecious, bearing separate male (staminate) and female (pistillate) flowers on the same plant. The inflorescence develops at the reproductive branches, rarely in the leaf axils on the upper part of the plant. The flowers have an indefinite structure called perianth or perigonium, consisting of five cream, yellow, greenish, reddish or purple tepals. The male flower is generally half the size of the female flower with a thin, straight and short pedicel, while the female flower is thick, curved and long. The basal disk of the male flower is divided into ten lobes, and ten stamens originate between them. Stamens with anthers are arranged in two whorls. The five external stamens are separated, longer than the inner ones, and join together on the top to form a set of anthers. The female flower has a ten-lobed basal disk, less lobulated than the male flower. The ovary is tricarpellary with six ridges mounted on the basal disk. The three locules contain one ovule each. A very small style is located on top of the ovary and stigma with three undulated, fleshy lobes (Alves, 2002).

In cassava, flowers are borne in a single branched panicle, with female flowers at the base and male flowers clustered towards the tip (Fig. 1a,b). The male flowers are more numerous than female flowers (Fukuda et al., 2002). Flowers usually begin to open around 10 AM and remain open for about one day (Ceballos et al., 2002). Cassava exhibits protogyny, and on a given branch, female flowers open first, and the male flowers open 10-15 days later. When male flowers open, the female flowers on the same branch are already fertilized or aborted. However, flowering on a single plant may last for more than two months, and both self- and sib-fertilization may occur, with the proportion of each dependent on the genotype, the environment, and the presence of pollinating insects (Kawano, 1980; Jennings and Iglesias, 2002). No genetic or physiological barrier prevents self-pollination in cassava. The intense endogamic depression and vegetative propagation act as a biological mechanism by which the high rate of heterozygosity of the species is maintained (Kawano et al., 1978; Kawano,1982).

Cassava is an outbreeding species possessing 2n=36 chromosomes and is considered an amphidiploid or sequential allopolyploid. In cassava, detailed karyomorphological studies showed haploid chromosomal complement with three functional nucleolar chromosomes. Six chromosomal types in duplicate suggest the allopolyploid nature of present-day cultivated types (Jos and Vasudevan,1989). Triploid (2n=54) and (2n=72) hybrids are also available in cassava. The meiosis in pollen and megaspore mother cells recorded regular bivalent formation with no abnormalities (Graner, 1935). Selection for high yield of tuberous roots and ideal non-branching plant type during domestication reduces seed fertility.

In cassava, the time and frequency of flowering are under genetic control and are strongly influenced by environmental factors like altitude and plant branching habit. For some clones, induction of flowering depends on long photoperiods up to 16-hour day length and temperatures of around 24°C (Keating, 1982; Alves, 2002). Cassava varieties exhibited wide variation in their flowering behaviour, which is positively correlated with the branching pattern of the genotype. Based on the flowering habit, the varieties are classified into non-flowering, sparsely flowering, moderate flowering, and profusely flowering genotypes. Generally, erect non-branching genotypes with high harvest index were selected as commercial varieties. However, the non-flowering nature of these genotypes limits their further utilization in breeding programmes. Hence, parents with a profuse flowering nature and extended periods of flowering were selected for initiating new cassava breeding programmes.

Fertility

The cassava pollen is generally yellow or orange and is large compared to other flowering plants. The pollen grains of cassava are quite large (90-150 micros) with 20-60% germination. The pollen size differs based on the ploidy level of the genotype. Cassava pollen loses

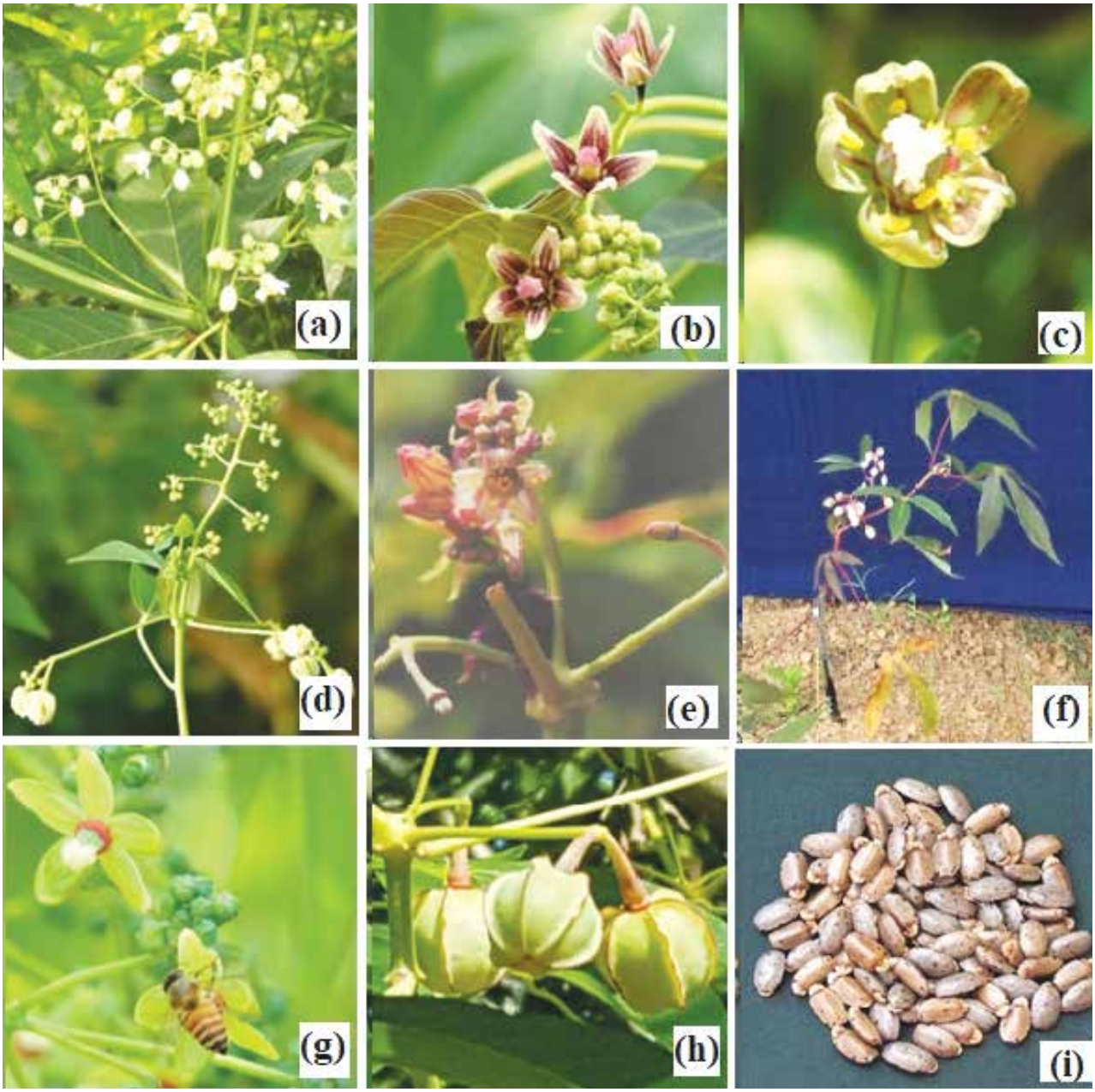

Fig. 1. Floral morphology of cassava: (a) Male flowers, (b) Female flowers, (c) Bisexual flower, (d) Male sterility, (e) Flowers of interspecific hybrids (*Manihot esculenta* X *M.caerulescens*), (f) Early flowering in inbreds, (g) Pollination of flowers by honeybees, (h) Fruits and (i) Seeds

viability rapidly after it is shed. Insects, mainly honey bees, generally cross-pollinate cassava (Fig.1g). The seed-setting ability of the cassava varieties has been reduced due to continuous vegetative propagation and directed selection for non-branching types. Fertility was highly variable, and the pollen's ability to promote seed set was unrelated to the fertility of the variety's female flowers (Jennings, 1962). Male sterility is common in cassava and among many varieties studied, 20 percent had deformed anthers and were male sterile (Cours, 1951). Pachytene pairing in relation to pollen fertility was studied in cassava clones with different levels of pollen fertility. Highly pollen-fertile clones showed regular pachytene and meiotic divisions. In the partially pollen-fertile clones, different pachytene abnormalities like non-pairing, deletion, duplication, and inversion were found in different chromosomes. The extent of pachytene abnormalities indicates the degree of cryptic structural hybridity in the cassava genomes (Jos and Vasudevan,1989).

Male and female cyathia have distinct developmental phases in cassava (Perera et al., 2012). Pollen viability was high during immature stages of plant development, and pollen mortality was common at later stages. Pollen

trimorphism in male gametophytes contributes to larger or smaller pollen size (Perera et al., 2012). The fruit and seed set were drastically reduced when flowers were covered for 2 or 3 days after anthesis day. The pollen tube growth rate is fast during the first 6 hours after pollination. The growth slows down after that, taking ten additional hours to reach the end of the beak. Although several tubes may reach the nucellar beak, only one was observed entering the embryo sac (Ramos et al., 2019). Genotypic and environmental factors and the manual manipulation of inflorescences and cyathia influence the timeline of fertilization. In cassava,early embryo rescue and ovule culture at 7 to 42 days after anthesis resulted in plants with increased levels of homozygosity up to 85.7%.

There is differential genotype-environment interaction on flowering among varieties, as well as a strong environmental effect on the number of flowering peaks within the same variety. Flowering (up to four blooms) was found to be associated with branching. Most flower buds formed at the early growth were found to be abortive. There is variation in flowering time (from four months to >eight months) and in the number of flowers between varieties. Late pollination enhanced seed set in less responsive clones. The uninterrupted availability of soil moisture and cool climate favoured early flowering and fruit set in cassava varieties (Ravi, 2005). Weather conditions influence flower opening; cloudy weather delays the opening of flowers, and capsules mature in 75 to 90 days (Fig.1a&b).

Self-pollination resulted in a low seed set of 33.15% against >80% in cross-pollination. Inbreeding led to the induction of bisexual flowers, male sterility, cleistogamy, and other floral variations in cassava (Fig.1c,d,e). Hybridization of cassava with wild species (*Manihot anomala* Pohl., *M.oligantha* Pav ubsp. Nesteli, *M. gracilis* Pax and *M. zehntneri* Ule) were fertile. Meiosis of the four species was regular with 18 bivalents and equal distribution of the 18 chromosomes during Anaphase I at each pole without any laggards, restitution nuclei or polyads (Nassar, 1978.). In interspecific hybrids of cassava with cera rubber, the random transmission of some of the parental chromosomal types through the male gametes of the interspecific hybrid was reported based on a karyotypic study (Magoon et al., 1971). The interspecific hybrids of cassava with *Manihot caerulescens* have bigger flowers (Fig.1f) and were found to be profusely flowering (Sheela et al., 2002). The fruit of cassava is an ovoid to ellipsoid, septicidal capsule (Oliveira and Oliveira, 2009), which dehisces two to three months after fertilization (Halsey et al., 2008). The seeds are carunculate, with abundant endosperm and an embryo with thin, flat cotyledons (Orlandini and Lima, 2014).

Polyploidy

In cassava, triploids recorded higher yield and starch content than diploids. Polyploidy seems to affect floral traits associated with premating barriers between polyploids and diploids (Pegoraro et al., 2016; Porturas et al., 2019). An increase in the length of pedicels accompanied the change from the diploid condition to the tetraploid condition. However, the initiation of flowering in tetraploids was delayed compared to the diploid counterpart. The triploids and tetraploids have larger flowers than the diploids. There was a significant reduction in pollen fertility and seed setting among the induced tetraploids compared to their diploid progenitors. The induced tetraploid showed irregular meiosis. At Metaphase-1 univalents, trivalents and quadrivalents were observed in addition to bivalents. In tetraploids, pollen sterility was very high (62-78%), presumably due to irregular meiosis, and the seed set was low. Triploids were produced by crossing diploids with the induced tetraploids(Sreekumari et al., 2000). The fruit set was comparatively high (20.5-34.3%)when diploids were used as the female parent. The pollen grains were highly sterile (>95.8%) in most triploid clones, ranging from 8.5 to 12.6%. Micropollen, a characteristic feature of triploids, is common. Fruit set and seed set were meagre when triploids were used as female parents. The occurrence of natural triploidization events has been reported (Sardos et al., 2009; Pillai et al., 2003) and it may also play a role in the evolution of new land races in cassava.

Manipulation of fertility

Cassava genotypes exhibited tremendous variation in their flowering and can be manipulated through mechanical/ exogenous application of plant growth hormones. The wild relatives of cassava are generally propagated through seeds. The highly fertile interspecific hybrids of cassava with closely related *Manihot* sp could be used as a donor parent in backcross breeding programmes to transfer seed fertility to the cassava varieties with reduced pollen and ovule fertility (Jennings, 1962).

Grafting/Pruning

The grafting technique is a viable tool for floral induction since it can promote the movement of mobile elements throughout the plant, such as water, nutrients, metabolites, and proteins (Mudge et al., 2009). It can transfer floral stimulus between different cassava genotypes. The initiation of floral development starts with the movement of florigen (signal), produced in the leaves and transported to the apical meristem through the phloem, where the interaction with other factors occurs (Amasino, 2010; Ceballos et al., 2017). The use of profusely flowering rootstocks could transfer the flowering stimulus to scions of non/sparsely flowering genotypes. Grafting in the early stages of plant

development increases survival and also favours the induction of flowering. High fruit set was found in less flowering genotypes when grafted onto highly flowering ones. An increase of 201% in the production of male flowers, 560% of female flowers, and 400% of fruits in BRS Formosa, a low flowering genotype grafted on a profusely flowering genotype, BGM0823 was reported. The grafted cassava plants recorded an increase in shoot production, although there was no change in the fresh root yield. Hence, the grafting of genotypes with high flowering rates can induce flowering in genotypes with low flowering rates (Souza et al., 2018). The effects of grafting have a genotypic dependency, which limits the potential for its generalised use for enhancing flowering in crossing nurseries in cassava breeding programs (Ceballos et al., 2017). Budding of sparsely flowering plants on profusely flowering genotypes also resulted in the induction of flowering in sparsely flowering cassava genotypes.

Pineda et al., (2020) reported that pruning young branches prevents the abortion of the first inflorescence, fostering seed production much earlier than in untreated plants of late flowering cassava genotypes. They advocated the combined use of extended photoperiod, pruning, and BA application to enhance flowering of late flowering genotypes such as CM 4919-1 and SM 3348-29, which otherwise produce very little or no seed. Besides, detopping of the shoot (30 cm from the top) of non-branching genotypes resulted in the induction of flowering in several low flowering cassava genotypes in India (9S174, Sree Jaya) of cassava (Darshan, 2017).

Plant growth regulators

The shift from the vegetative to the reproductive stage depends on both endogenous (hormones) and environmental (temperature and photoperiod) signalling for the differentiation of the apical meristem into floral meristem (Bernier and Périlleux, 2005). This change in the apical meristem can be induced by the exogenous application of growth regulators such as auxins, gibberellins, abscisic acid, and ethylene (Yang et al., 2016). The effect of plant growth regulators (PGRs) to regulate plant reproductive development has been researched in many plant species (Rademacher, 2015).

Indira et al., (1977) attempted exogenous application of growth regulators to induce flowering in cassava. Indole acetic acid (IAA), Naphthalene acetic acid (NAA), and ascorbic acid (AA) had a positive effect in promoting flowering in cassava as compared to other growth regulators like TIBA, Ethrel, and Kinetin. IAA (50ppm) and AA (100ppm) treated plants of a shy flowering landrace S1315 resulted in a higher number of female flowers and a higher fruit setting percentage. Application of ascorbic acid @100 ppm resulted in the initiation of flowering in the fifth month, followed by treatment with NAA @ 50 ppm. Ascorbic acid @ 100 ppm induced a higher number of flowers in sparsely flowering cassava varieties. The nutritional conditions affect cassava flowering behaviour, and flower/fruit production was significantly enhanced in the unfertilized (NPK) treatment across four different genotypes (Pellet and El-Sharkawy,1993). Induction of flowering *in vitro* plants by adding growth regulators *viz.*, gibberellins and cytokinin in the presence of auxin has also been reported (Tang et al.,1983).

The ethylene signalling affects the floral development in cassava. The effect of the anti-ethylene plant growth regulator, silver thiosulfate (STS), on mitigation of ethylene's effects on cassava flower development was reported. STS did not affect the timing of flower initiation but enhanced the development of early inflorescence, flower development, flower longevity and increased flower numbers (Hyde et al., 2019). Studies on silver accumulation and treatment localization support the hypothesis that the beneficial effects of STS are confined to tissues of the shoot apex. The most effective timing of application was before inflorescence appearance.The additional spraying of benzyl adenine in pruned plants also promotes flower development and often results in the feminization of male flowers (Pineda et al.,2020).

Photoperiod

Pineda et al., (2020) studied the effect of extended photoperiod on flowering of cassava varieties. Five genotypes with contrasting flowering behaviour were grown in dark and extended photoperiod conditions for three seasons. Extended photoperiod was achieved with different red light-emitting diodes (LEDs) with 62-635 nm wavelength all night long or through night breaks. Extended photoperiod reduced height and number of days to first branching, particularly in non- or late-flowering genotypes. They reported that a minimum of 0.02 $\mu molm^{-2}s^{-1}$ was required to induce earlier flowering in plants illuminated all night. Extended photoperiod promoted earlier flowering in erect-plant genotypes of cassava. They recommended the installation of 50W LED lamps, fixed at 3 m above ground in a 4.5 m grid in pollination nurseries to extend photoperiod in cassava for accelerating the breeding programmes.

Expression of floral genes in cassava

Studies on the model plant *Arabidopsis* have helped to understand the regulatory role of various gene networks involved in floral transition in plants (Srikanth and Schmid, 2011; Wellmer et al., 2014; Chen et al., 2018). The genes *CONSTANS (CO), FLAVIN KELCH F BOX 1 (FKF1), FLOWERING LOCUS T (FT)* and *GIGANTEA (GI)* have key regulatory role in determining floral transition in *Arabidopsis* (Fowler et al., 1999; Fowler et al., 1999; Andrés and Coupland, 2012). In many plant species, flowering is induced by the synthesis of the mobile

protein, *FT* in leaves (Andrés and Coupland, 2012; Chen et al., 2018). The *FT* locus is highly conserved among the flowering plants. Thus, overexpression of *Arabidopsis AtFT* in cassava induces early flowering within four to five months of planting in the transgenic cassava lines (Bull et al., 2017). Pollination of pistillate and staminate flowers from clonal propagates resulted in the development of viable seeds in cassava (Bull et al., 2017). Developing cassava with heritable allelic edits but lacking foreign DNA ie the T-DNA encoding the genome editing tools, requires the generation of a segregating population in which the T-DNA can be crossed out. Cassava seldom flowers in glasshouse conditions, but expression of *AtFT* in the glasshouse-grown transgenic cassava permitted rapid flowering and heritability of edited lines (Bull et al., 2018). This shows the potential scope of including genetic engineering technology in speeding up floral transition in cassava breeding programs. The cassava genome encodes for two *FT* genes *MeFT1* (Manes.12G001600.1) and *MeFT2* (Manes.13G000800.1) (Adeyemo et al., 2019). Expression studies showed the modulation of the expression pattern of MeFT2 in response to photoperiod (Adeyemo et al., 2019). Both *MeFT1* and *MeFT2* genes displayed higher expression in early flowering cultivars, suggesting their functional role in regulating flowering in cassava (Adeyemo et al., 2019). Further studies on the functional characterization of *MeFT1* and *MeFT2* would help to understand the molecular mechanism of the genes for manipulation through CRISPR-mediated breeding.

Behnam et al., (2021) found that cassava shares conserved genes for the photoperiodic flowering pathway, including florigen, anti-florigen and its associated transcription factor (*GIGANTEA, CONSTANS*, *FLOWERING LOCUS T*, *CENTRORADIALIS/TERMINAL FLOWER1* and *FD*) and florigen downstream genes (*SUPRESSOR OF OVEREXPRESSION OF CONSTANS*1 and *APETALA1/ FRUITFUL*). In cassava, flowering was induced in the dry season in the mountain regions, and flowering time was found to be correlated with the expression of *MeFT1*, and homologs of *Arabidopsis GI*, *PHYA*, and *NF-Y*s (Tokunaga et al., 2022) and can be a survival mechanism during moisture stress.

Cryo-conservation of cassava pollen

As described by Engelmann (2004), cryopreservation is the conservation of live tissues at hyper-low temperature (-196°C), mostly that of liquid nitrogen, which is an important technique currently available to guarantee the safe, cost-effective, and long-term conservation of germplasm. All metabolic processes and cell divisions are arrested at this condition (-196°C). Hence, the live materials stored in this condition can be conserved without any change for a hypothetically limitless period of time.

The cryo conservation of pollen can potentially overcome challenges of breeding programs, such as flowering asynchrony between different parent genotypes, and the production of insufficient pollen in nature. Cryopreservation of pollen grains to conserve nuclear genetic diversity is desirable in horticultural crops for various reasons. Pollen grains, which are cryopreserved can be a significant approach for the crossing of elite plants particularly in crops where flowering is difficult, production of hybrid seeds, development of pre-breeding lines, germplasm exchange, biotechnological and other basic studies. The objective of the development of a protocol for pollen cryopreservation is to collect matured viable pollen from desirable plants and treat it to retain its viability, germination ability, and ability to fertilize the ovule in natural condition after storing them in liquid nitrogen (Hanna and Towill, 1995). Cryopreserved pollen allows plant breeders to plan hybridization programmes across geographical and seasonal limitations (Ganeshan et al., 2007). Pollen cryobank ensures year-round availability of pollen for biology, biotechnology, and other research programmes (Shivanna, 2003). Cryopreserved pollen grains also serve as conservation of genetic diversity where it is difficult to preserve diversity as live plants and seeds.

The crossing among the lines/varieties of cassava and later selection (mass selection) based on phenotype to identify elite plants within the F_1 progenies and subsequent clonal generations is the widely applied method of developing new varieties in cassava (Ceballos et al., 2004). The hybridisation between the elite lines is very difficult in cassava since the flowering is dependent on environmental conditions and the genotype. Lack of synchronization of flowering between the parents is the major drawback in cassava breeding. This type of flowering behaviour slows down cassava improvement through breeding. In such contexts, it is convenient to store pollen from the desired male parent for later use in hybridisation with the female parent when the female flowers are available. The low fertilization rate in cassava could be due to the low pollen viability reported for *Manihot* species, because pollen lasts for only 48 hours after anthesis (Halsey et al., 2008). According to Vieira et al., (2012), the pollen remains viable for six days when conserved in calcium chloride, and loses its viability when stored fresh at room temperature. Thus, the difficulties in the breeding of cassava can be overcome by the use of stored pollen.

The detailed protocols for pollen cryopreservation of cassava have been given by Vivek et al., (2019a). The male buds were bagged one day before anthesis and collected on the next day morning between 09.00 AM and 10.30 AM. Freshly collected male flowers were placed in a petri dish lined with moist paper and brought immediately to the laboratory. The collected flowers were stored in liquid nitrogen after placing them in the cryovials.

The stored cassava pollen samples were tested for viability using acetocarmine staining, *in vitro* germination tests, andwas also used for hand pollination. In the staining test, pollen grains were stained with acetocarmine and observed under the microscope. Deeply stained pollen grains were classified as viable, while those with abnormal size and stained in a light colour were considered non-viable. A non-significant difference was observed between the varieties for the viability of fresh pollen in the acetocarmine staining test. In contrast, a significant difference was observed for *in vitro* pollen germination and fruit set in the field conditions. Similarly, Dutta et al., (2013) reported that the *in vitro* pollen germination test was reliable in the mango pollen viability study, whereas acetocarmine tests obtained overestimated results. Chaudhury et al., (2010) and Shivanna and Helpson-Harrison (1981) also observed the over estimation of viability in fresh and stored mango pollen.

Medium for pollen germination under *in vitro* conditions was standardised in several crops like mango, citrus, gladiolus, rose, tomato (Ganeshan et al., 2007), and taro (Mukherjee et al., 2016). Germination of cassava pollen under *in vitro* conditions was achieved using the sitting drop method. A drop of pollen germination medium containing 5% sucrose, 300 mgl^{-1} calcium nitrate, 200 mgl^{-1} magnesium sulfate, 100 mgl^{-1} boric acid, and 100 mgl^{-1} potassium nitrate (Mary et al., 2015) was laid on the microscopic slide and pollen was brushed. This was incubated at room temperature for six hours, and through an optical microscope, germinated pollen grains were recorded. Stored pollen was used to test their fertility/crossability for controlled field pollination. The hand pollination was done by placing cryopreserved pollen onto the receptive stigma and pollinated female flowers were immediately protected from insects with muslin cloth. Cryopreserved cassava pollen for 45 days recorded 63.9% and 59.1% staining as well as 51.0% and 49.5% *in vitro* germination in Vellayani Hraswa and Sree Padmanabha (Fig. 2) and the fruit set recorded was 47% and 46.7%, (Fig. 3) respectively (Vivek et al., 2019b). Both staining percentage and *in vitro* pollen germination were reduced to zero at later stages of storage. Cassava pollen is highly susceptible to desiccation, and there is rapid moisture loss and reduction in viability when stored at room temperature. Vieira et al., (2015) kept the cassava pollen at room temperature and observed total loss of pollen viability after 24 hours. Daniel (2011) observed poor pollen survival in West African yams with drying and suggested the 'wet-cold' storage procedure.

The pollen once stored at a temperature below -160°C would theoretically have an infinite period of longevity (Stanwood, 1985). Mukherjee et al., (2016) studied pollen cryopreservation in taro and observed that the degree of successful fruit set in taro with cryo-stored pollen was on par with conventional fruit set. In wild

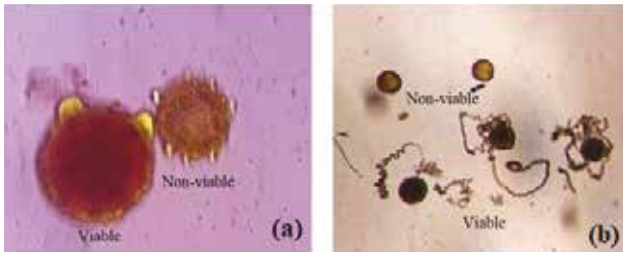

Fig. 2. Viability of cryopreserved cassava pollen assessed by acetocarmine test and *in vitro* germination tests (a) pollen staining and (b) germinating pollen under *in vitro* condition (Source: Vivek et al., 2019a)

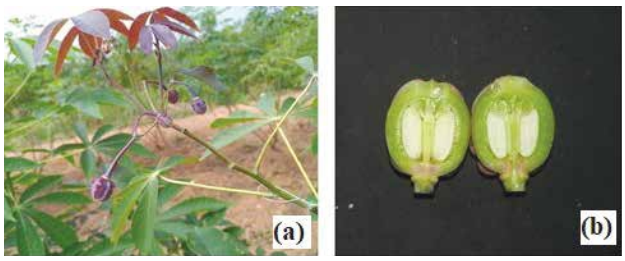

Fig. 3. (a) Fruit set by the cryo-stored pollen and (b) Developing seed pollinated by the cryo-stored pollen

pineapple accessions, Silva et al., (2017) achieved 62.67% *in vitro* pollen germination and 70.83% seed formation with cryopreserved pollen grains. Significantly higher pollen viability of three Indian mango cultivars was reported by Dutta et al., (2013) when stored at -196°C by different pollen viability tests such as acetocarmine, fluorescein diacetate (FDA), and *in vitro* germination. Lora et al., (2006) efficiently stored cherimoya (Annona cherimola Mill.) pollen at −196°C (liquid nitrogen). Similarly, Alba et al., (2011) conserved pollen from 12 olives (*Olea europaea* L.) cultivars for 1 year in liquid nitrogen. The highest mean percentage of fruit set in cassava was obtained when pollination was carried out between 11.30AM to 12 noon. The fruit set percentage was reduced when pollination was carried out before and after the peak receptivity period (11.30AM-12noon) of the stigma (Vivek et al., 2019a). In cassava, many non-branching genotypes with suitable plant type flowered rarely. Even though flowering can be induced through de-topping and hormonal treatment, the number of flowers and flowering peaks will be less. However, the cryopreservation of the rare pollen will help storage and need-based use of pollen in cassava breeding programmes. It also helps in an international cassava pollen exchange, strengthening the collaborative inter-institutional breeding programmes.

In cassava, combining pruning young branches and spraying BA allowed the production of more seeds from erect cassava genotypes in a short period. The implementation of these procedures along with cryo preservation will improve the breeding efficiency in cassava (Pineda et al., 2020).

Breeding success in India

ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India, initiated the cassava breeding programme in 1963. A total of 1216 accessions were conserved in the field gene bank at the National Repository of Tuber Crops germplasm at ICAR-CTCRI, Sreekariyam, Kerala, India. Cassava breeding in India was focused on developing varieties for table purpose as well as for industry and selection parameters were set accordingly. Thirty-three cassava varieties were released in India through different cassava breeding programmes. The developed varieties include short duration (6 months), high starch triploids, nutrient efficient, and cassava mosaic disease-resistant varieties. The improved varieties were generally developed through hybridization, followed by clonal selection. Most of the released varieties released are diploids. Three triploid hybrids, Sree Harsha, Sree Athulya and Sree Apoorva with high extractable starch content (30-34%) were released through a triploidy breeding programme. Recently, cassava mosaic disease (CMD) emerged as the major threat to cassava cultivation in Southeast Asian countries. Five high-yielding varieties *viz*., Sree Reksha, Sree Sakthi, Sree Suvarna, PDP-CMR1 and Sree Kaveri with resistance to CMD caused by Indian Cassava mosaic virus and Sri Lankan cassava mosaic virus were also released in India. All these breeding successes in cassava have been brought through the successful manipulation of flowering and the development of the pre-breeding population in cassava.

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