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Grafting as a strategy to increase flowering of cassava

Leonardo Silva Souza[a], Rafael Parreira Diniz[a,b], Reizaluamar de Jesus Neves[a], Alfredo Augusto Cunha Alves[b], Eder Jorge de Oliveira[b,*]

[a] Universidade Federal do Recôncavo da Bahia, Campus Cruz das Almas, 44380-000 Cruz das Almas, BA, Brazil
[b] Embrapa Mandioca e Fruticultura, Rua da Embrapa, Caixa Postal 007, 44380-000 Cruz das Almas, BA, Brazil

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ABSTRACT

In cassava (*Manihot esculenta* Crantz), transferring genes via genetic breeding depends on crosses between contrasting progenitors, which is often limited by the low flowering rate of many genotypes. The main purpose of this work was to evaluate the effect of grafting on floral induction of cassava. For this, three genotypes were used: 1) BRS Formosa: a genotype with low flowering rate; 2) BGM0823: a genotype with high flowering rate; and 3) FLA05-02: a genotype of *M. esculenta* ssp. *flabellifolia* with high flowering rate. Cleft grafting was performed to generate the following treatments: Self-grafting of: 1) BGM0823 (Self-0823); 2) BRS Formosa (Self-Formosa); and 3) FLA05-02 (Self-FLA); and grafting of the genotypes, with the first being the scion and the second the rootstock: 4) BGM0823 × BRS Formosa; 5) BGM0823 × FLA05-02; 6) FLA05-02 × BRS Formosa; 7) FLA05-02 × BGM0823; 8) BRS Formosa × BGM0823; 9) BRS Formosa × FLA05-02; and also ungrafted treatments: 10) BGM0823; 11) BRS Formosa; and 12) FLA05-02. The results showed a 201% increase in the production of male flowers, 560% of female flowers and 400% of fruits in BRS Formosa grafted on BGM0823. BGM0823 (rootstock) also increased fruit production by 190% of FLA05-02. The grafted cassava plants exhibited an increase on the shoot production, although there was no change in the fresh root yield. The grafting of genotypes with high flowering rates can induce flowering in genotypes with low flowering rates.

1. Introduction

Cassava (*Manihot esculenta* Crantz) has gained importance in recent decades due to its many forms of use: as human food, in which it is considered a food security crop and is part of the diet of 800 million people in the tropics (El-Sharkawy, 2012); as animal feed; and for various industrial uses, including to make biofuels. Therefore, cassava breeding programs in Brazil and other countries in Latin America and Africa have increased their research with the objective of obtaining superior genotypes that meet the specific demands of farmers and industries.

For the selection of high performance genotypes, it is necessary to increase the genetic variability, which can be done through hybridization or self-fertilization in order to produce seeds. However, among the major drawbacks of this process is the absence or reduced flowering rate observed in most genotypes and the lack of synchronization of flowering (Ceballos et al., 2017). The production of fertile flowers is a basic premise for plant breeding, but obtaining flowers in a synchronized manner and within a suitable time interval is a major challenge (McGarry et al., 2017), considering that some genotypes do not produce flowers (Ceballos et al., 2017).

Erect and late-branching plants are preferred by farmers due to the greater ease of crop handling and possibility of using mechanized planting systems. In general, these plants do not bloom or bloom poorly during the normal growing cycle. Thus, efforts to develop new cassava genotypes to meet the demands, especially for mechanized planting, tend to select plants that flower late and little. In other words, the use of erect or late-branching genotypes as progenitors in recurrent selection cycles results in limited production of seed, so it is necessary to search for alternatives to induce flowering.

Some physiological aspects help explain the difficulties of flowering of some genotypes. The transition from the vegetative to the reproductive stage depends on both endogenous (hormones) and environmental (temperature and photoperiod) signaling, determined by the differentiation of the apical meristem, where the cells undergo changes in development (Bernier and Périlleux, 2005). This change of 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). In addition, flowering is initiated from the floral signs, also called floral or florigen stimulus. Amasino (2010) studied these floral signals at the molecular level and found that the overexpression of the FT (florigen) gene accelerates the flowering rate

* Corresponding author.
E-mail address: eder.oliveira@embrapa.br (E.J.d. Oliveira).

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in *Arabidopsis thaliana* plants. In turn, Adeyemo et al. (2011) characterized the EFL4 gene (MeELF4) and identified the circadian genes of the cassava photoperiod, because the plants responded to diurnal rhythms or photoperiodic changes and the MeELF4 gene exhibited its expression at dusk in several parts of the plant.

The floral signals are produced in response to day length and translocated from the leaves to the apical meristem, where the flower bud production is established (Corbesier and Coupland, 2005). The hypothesis that florigen can move from leaf to meristem was demonstrated in *Arabidopsis thaliana* and rice (Corbesier et al., 2007; Tamaki et al., 2007). In these studies, the results showed that most if not all changes associated with the transition from vegetative to reproductive stage induced by day length could be ascribed to florigen.

Considering that florigen moves from the leaf to the meristem, Ceballos et al. (2017) evaluated floral induction in cassava by grafting. Initially, grafting was performed using late blooming or non-flowering genotypes as scions and genotypes with high flowering rate as rootstocks, and it was found that none of the treatments produced branches or flowers. In a second step, after the development of the scions, their cuttings were planted in the field, where three phenotypic responses were observed: some genotypes did not branch and did not flower; other genotypes branched but produced no flowers; and finally, in one genotype there was branching with abundant production of flowers, fruits and seeds in relation to ungrafted stem plants. Therefore, the grafting technique is a viable tool for floral induction, since it can promote the transfer of mobile elements throughout the plant, such as water, nutrients, metabolites and proteins (Mudge et al., 2009). The knowledge that the initiation of floral development starts with the movement of florigen (signal), which is produced in the leaves and transported through the phloem to the apical meristem where the interaction with other factors occurs (Amasino, 2010; Ceballos et al., 2017), is an advance in the analysis of floral induction in cassava. In this sense, the use of abundant flowering rootstocks could transfer the flowering stimulus to scions that show low flowering rate.

In addition to grafting, other floral induction techniques have been mentioned in the literature, such as the insertion of a flowering promoter sequence by inoculation of the zucchini yellow mosaic virus in melon (*Cucumis melo* L.) (Lin et al., 2007) and floral induction in cotton after inoculation of the cotton leaf crumple virus (McGarry and Ayre, 2012). In cassava, new techniques have demonstrated the over-expression of *Arabidopsis* FLOWERING LOCUS T in *Agrobacterium*-mediated transformed cassava, triggering early flowering in glasshouse-grown plants (Bull et al., 2017). These authors reported success in the expression of the inserted sequence, so that the transgenic plants emitted flowers and produced fruits from four months after planting. Although floral induction in cassava can be done using different techniques, few institutions have the necessary mastery of gene manipulation to create, edit, and insert promoter sequences into the cassava genome. In addition, the use of transgenic cassava, especially in Brazil, is not yet regulated, so it will undergo a long process of standardization and biosafety studies, especially considering that the species is native to Brazil, where simple and efficient techniques to induce flowering should still be considered. Therefore, the objective of this work was to analyze the possibility of flower induction in cassava plants through flowering signal transmission using different scion-rootstock combinations of genotypes with low or high flowering rate.

2. Materials and methods

The experiments were carried out in two stages (nursery and field) at the experimental area of Embrapa Mandioca e Fruticultura (Embrapa Cassava & Fruits research unit) in Cruz das Almas, Bahia, located at coordinates 12°40′19″S and 39°06′22″W, 220 m above sea level, from February 2015 to April 2016.

2.1. Plant material

Three genotypes belonging to the Cassava Germplasm Bank (CGB) of Embrapa Mandioca e Fruticultura were selected: 1) BRS Formosa (*M. esculenta*), an elite genotype introduced by Embrapa that has a low flowering rate; 2) BGM0823 (*M. esculenta*), a genotype of the CGB that has a high natural flowering rate; and 3) FLA05-02, a genotype of *M. esculenta* ssp. *flabellifolia*, a subspecies that has a high flowering rate. *M. esculenta* ssp. *flabellifolia* was also selected due to the potential of interspecific crosses with *M. esculenta*, which is currently attracting the interest of breeders, especially for the introduction of resistance to pests and diseases.

In February 2015, about 200 stem cuttings (10–12 cm length) of BRS Formosa and BGM0823 were planted in polyethylene bags (10 cm × 25 cm, diameter × height), filled with potting mix containing soil, vermiculite and chicken manure (2:2:1, v:v). At the same time, about 400 open-pollinated seeds of the genotype FLA05-02 obtained in the CGB were planted in bags similar to those used for the cuttings. The planted materials were placed in a greenhouse with 50% shade, where the temperature and relative humidity varied from 20 to 35 C and 50 to 80%, respectively, during the period of seedling production.

2.2. Grafting

The choice of grafting method was based on a pilot test carried out previously. In this test, three types of grafting were used: cleft grafting; splice grafting and normal “T” type budding. The graft survival rate was evaluated considering the production of seedlings with apparent adherence and good vigor. The cleft grafting presented higher survival rate and was used to perform grafts with different scion-rootstock combinations of the three selected genotypes. In summary, when the plants’ stems reached a diameter of approximately 6 mm, the seedlings were cut at medium height, leaving a pair of leaves in the rootstock at a height of approximately 12 cm (Fig. 1).

Then, we selected a stem with diameter similar to the rootstock for fork formation (grafting), and a longitudinal slot was opened (1.0 cm) in the rootstock. The scion, cut into a "V" shaped wedge, was inserted into the rootstock carefully to align with the cambial zone and secured with a small plastic clamp, and bagged to maintain high moisture (Fig. 1). Two leaves of the rootstock were maintained until the development of new leaves by the grafted part, which occurred approximately 15 days after grafting, when they were manually removed.

The rootstocks and scions produced by the vegetative propagation of the BRS Formosa and BGM0823 genotypes reached the grafting stage at 25 days after sprouting, while the rootstocks and scions of seminal origin (FLA05-02) reached the time for grafting 30 days after sowing. At the end of the grafting process, 12 treatments were obtained for the flowering analyses: 1) self-grafting of BGM0823 (Self-0823); 2) self-grafting of BRS Formosa (Self-Formosa); 3) self-grafting of FLA05-02 (Self-FLA); 4) BGM0823 as scion × BRS Formosa as rootstock (0823/Formosa); 5) BGM0823 as scion × FLA05-02 as rootstock (0823/FLA); 6) FLA05-02 as scion × BRS Formosa as rootstock (FLA/Formosa); 7) FLA05-02 as scion × BGM0823 as rootstock (FLA/0823); 8) BRS Formosa as scion × BGM0823 as rootstock (Formosa/0823); 9) BRS Formosa as scion × FLA05-02 as rootstock (Formosa/FLA); 10) BGM0823 ungrafted BGM0823 (0823); 11) BRS Formosa ungrafted (Formosa); and 12) FLA05-02 ungrafted FLA05-02 (FLA).

The plastic bag and clamp used to protect the grafting union were removed seven and twelve days after the grafting procedure, respectively. After grafting, the treatments were kept in a greenhouse, irrigated via micro sprinkler, for about 80 days.

2.3. Evaluation of the development of plants in the nursery

To verify the viability of the grafting, an experiment was performed with all treatments. The treatments were set up in a completely