

TIPS FOR SUCCESSFUL CROSSES IN AMARANTHS

Amaranthus cruentus (L.)

Background

Amaranth is one of the economically important traditional leafy vegetables in many countries, and it is reported that the leaves are consumed in many communities in sub-Saharan Africa (Sogbohossou *et al.*, 2015; Dinssa *et al.*, 2016). In South and North America, It has been grown for thousand years for its grains (Stetter *et al.*, 2016). Besides, a shift was reported from household to commercial production of Amaranth in some regions, where the local demand is more led by higher-yielding varieties of excellent colour, taste, and shelf life (Dinssa *et al.*, 2019). The development of pure lines or hybrids is, therefore, an utmost need and a real advantage for large scale cultivation and commercialisation of Amaranths for leaf and grain yield purposes.



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Overview on the flower development

The structure of *Amaranthus* spp inflorescence is composed of unisexual flowers that are developed in numerous dense clusters. The inflorescence is dense and abundantly branched in numerous raceme or spikes, especially semidropping for *Amaranthus cruentus*. Each cyme has a determinate principal axis with a terminal male flower, followed by pairs of opposite or occasionally single lateral branches of females' flowers. Individual flower clusters or glomerules are developed alternately along the principal axis (Fig.1). This species is a monoecious plant with basically significant anemophily (Schippers, 2004). There is, therefore, a wide range regarding the outcrossing rate, which varies from the initial to the first generations between 0.10 and 0.50, showing then a high percentage of autogamy of the plant (Hauptli and Jain, 1985).



Figure 1: Inflorescence of *A. cruentus* consisting of flower clusters wherein a male flower in the centre is surrounded by several female flowers (Copyright: GBioS)

1 2 3 Important steps for crossing in Amaranths

Four crossing methods are commonly used: open pollination, hot water emasculation, hand emasculation, and auto-pollination. All four methods produce successful crosses, but the success rates and variances are different from one to another. The choice of the plants before crossing is based on the flower status at the initial phases (depending on the genotype, or flowering synchronization using day length) when the male flowers with the stamens are not yet visible on the inflorescence. It is suitable to carry out the hot water and hand emasculation in a greenhouse or a screen house where there is no pollinator or wind.

METHOD 1. OPEN POLLINATION

The open pollination is made between two plants under a single bag without emasculation of the female parent. During that method:

- Step 1.:** Leaves around the inflorescence of the female and the male plant are removed to prepare the crossing.
- Step 2.:** Female and male crossing partners are attached to each other.
- Step 3.:** Single partners are isolated with pollen proof bag to avoid contamination by foreign pollen.

METHOD 2. HAND EMASCULATION

Amaranth's hand emasculatation is the most complex, due to the smallness of the flowers to emasculate. In fact, each male flower has enough pollen to pollinate all the plant. In addition, it is critical to remove all male flowers from the plant until its dehiscence. The procedure is similar to open pollination and the stamens appearance should be considered to emasculate at the right frequency until the flowers dehiscence. It is therefore advisable while crossings some species such as *Amaranthus cruentus* to emasculate the flowers on diurnal basis. This method is time-consuming owing to the time of emasculatation. However, its success rate is the highest of the three methods (Stetter et al., 2016). A small label indicating the date of pollination and the cross can be put on the peduncle of a single flower.

METHOD 3. HOT WATER EMASCULATION

This crossing method is characterized by hot water treatment applied to the female parent (Fig.2). According to Stetter et al. (2016), this method has a high potential if the key conditions for a successful application can be identified. To achieve approximately a high rate of success, the flowers are sterilized at 45°C during 10 min but an adaptation of temperature to 47°C during 10 min until 15 min may contribute to a higher rate of success.



Figure 2: Hot water emasculatation by water bath (left). Female inflorescence soaked into the water bath (right).

For all crossing methods female and male plants are bound to stay in the same pollination bag. The bag is shaken once in a while to facilitate pollen transfer. Sometimes, an alternative is to collect the pollen from the male plants using a brush before pollination (Fig.3). Eventually, two methods are used to evaluate the Success rate of the crosses. The first way requires morphological markers such as seeds colour or stems colour to identify the putative hybrids. Otherwise, molecular markers which are more accurate can be used for hybrids determination identification and work out the rate of success.



Figure 3: Collecting pollen from a male plant (Abakaliki, Nigeria).

METHOD 4. AUTO- POLLINATION

For Auto-pollination of Amaranths, it is firstly necessary to identify and label the inflorescence at flowering initiation when the stamens are not yet visible. It is often better to remove the leaves around the inflorescence before covering them with a pollination bag. Its role is to avoid foreign pollen transfer (Fig.4). After 10 to 14 days, the bag of pollination can be discarded and the seeds are harvested from the inflorescences and kept in small tea bags tagged (Fig.5). During this time the main aspect is colour change of the inflorescence (green to brown, red to pink) and the seeds depending on the genotypes.



Figure 4: Amaranths flowers protected with pollination bag during seed multiplication (Abakaliki, Nigeria).



Figure 5: Seeds collection, (1): envelop and its content poured in a plastic receptacle, (2): seeds and waste, (3): after blowing the content, seeds are poured in a small bag, (4): the small bag is tagged (Abakaliki, Nigeria).



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