Ensuring the Success of Pigeonpea Hybrids by Focusing on Purity of Parental Lines and Appropriate Management Practices

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of the traits, obcordate leaf, is known to be due mainly to one gene which is recessive in nature (Fig 2). This obcordate leaf trait is expressed at around a month after planting, thus this trait is an ideal candidate to introgress into A and B lines to ensure the purity of those inbred lines. Some progress has already been done on this direction. Further cycles to incorporate the obcordate trait in pure A and B lines and develop hybrids by crossing with the R lines will be done.

Two additional promising morphological markers or hybrids will be studied: sesame leaf and deep coloration of the stem. The inheritance of these traits will be evaluated and subsequently breeding schemes will be implemented to introgress these traits into appropriate parental lines to be used in combination with the previously mentioned obcordate leaf trait.

Fig 2: Purity of parental lines and hybrids using NEPs
1. Introduction

Pigeonpea [Cajanus cajan (L.) Millsp.] is an important legume crop, grown mainly in the semi-arid tropics of Asia, Africa, Latin America, and the Caribbean. The total world area planted with pigeonpea is 4.6 million ha. India is the number one producer (3.5 million ha) of pigeonpea; despite this, there is not sufficient amount available to meet the nutritional needs of the large and mainly vegetarian resource-poor population and importation of pigeonpea from other countries like Myanmar and parts of Africa is needed to fulfill the country needs. Although dozens of pigeonpea varieties have been released, the productivity of pigeonpea has remained stagnant at around 700 kg ha\(^{-1}\). This is mainly due to various genetic, management, abiotic, and abiotic constraints. Since the area of pigeonpea cultivation is not likely to be increased, the emphasis moves to break the yield gap and to increase productivity by genetic enhancement of germplasm by incorporating tolerance to biotic and abiotic stresses, and by replacing the traditional varieties with the newly developed options.

The commercial success of hybrids generally depends on the consistency of performance and genetic purity of seeds plays an important role in the realized yields. The genetic purity of hybrids depends on a number of factors such as availability of pure inbred parental stocks (A, B, and R lines) and appropriate management practices including critical components of isolation distance and timely roguing. The seed producing farmers and the seed industry in general should follow high quality standards to produce pigeonpea hybrid (A x R) seed otherwise the great promise of hybrid pigeonpea will be in peril.

nuclear and mitochondrial fingerprints of the commonly used A, B, R and released varieties. Validation studies will be done by testing the selected nuclear and mitochondrial markers on artificially generated mixtures of A, B, R and common varieties in different combinations. Selected nuclear and cytoplasmic molecular markers will be used to create a pigeonpea nuclear and cytoplasmic fingerprinting kit. Samples of parental lines and hybrid seed from selected farmers will be tested using various sampling techniques to evaluate the detection capacity of the molecular markers selected of the nuclear and cytoplasmic fingerprinting kit.

4.3 Development and application of NEP (naked - eye polymorphism) purity tests

The use of distinctive morphological traits, easy to identify by naked eye during early growth stages and not present in cultivated varieties or hybrids, could offer a great tool to ensure purity of parental lines and hybrid seed with minimum cost. A few morphological traits have been identified and could be incorporated into A, B and/or R parental lines to explore this option. One.

Fig 1: Genetic purity test for hybrids and parental lines (A and R).
2. Purity Maintenance of Parental Lines

Availability of the pure (true to type and inbred) parental lines seed is a prerequisite to be able to start hybrid seed production. In the case of A line (male sterile, C. cajanifolius – wild relative - cytoplasm and C. cajan – cultivated-nucleus) proper field isolation (more than 500 m from another pigeonpea field) and presence of pollinating bees should be taken into account; alternatively nets where artificial (manual labor-based) or bee-aided pollination takes place could also be used. A and B (male fertile, C. cajan – cultivated-nucleus and cytoplasm) lines should be planted using recommended ratios i.e. 3 A plants to 1 B plant. A map of the field should be developed at planting time and each row should be labeled as A and B and the labels should remain in the field until harvest is completed. Recommended spacing is 90 cm between rows and 30 cm between plants within a row should also be used to maximize yield of the A line. The A line is maintained/increased by crossing the A line (male-sterile) acting as female with the B line (maintainer) used as male. Proper identification of A lines while increasing A line seed should be done to avoid the presence of off-types, partial fertile plants and diseased plants. Thus regular inspections and rouging should take place before flowering. Synchronization of flowering between the A and B line is also important to maximize seed production. The B line should be harvested first to avoid mixtures with seed from the A- line. A- and B- line seed should be stored separately. A line seed will be used for hybrid seed production.

In the case of R-line (restorer) increase, proper field isolation (more than 500 m) should be used. The presence of bees is optional since both selfing and out-crossing within the same genotype would not alter purity. Alternatively selfing could be done under net, no insects required. Recommended spacing of 90 cm between rows and 30 cm between plants within a row is recommended to maximize yield. Regular field inspections and removal of off-types and diseased plants should also be done.

3. Hybrid Seed Production

Proper field isolation (more than 500 m from another pigeonpea field) should be used and presence of pollinating bees should be ensured; alternatively nets where artificial (manual labor-based) or bee-aided pollination takes place could also be used. Pure A and R lines should be planted using recommended ratios, i.e. 3 A plants to 1 R plant. A map of the field should be developed at planting time and each row should be labeled as A and R and the labels should remain in the field until harvest is completed. Recommended spacing is 90 cm between rows and 30 cm between plants within a row. The A line (male sterile) will act as female and the R line (restorer) as male to generate hybrid seed on the A plants. Proper identification of A lines during hybrid production should be done to avoid the presence of off-types, partial fertile plants and diseased plants. At the same time the R lines should be inspected and off-types and diseased plants should also be removed. Thus regular inspections and rouging should be done. Synchronization of flowering between the A- and R- line is important to maximize seed production. The R- line should be harvested first to avoid mixtures with produced on the A line (in this case hybrid seed). The seed harvested from A - lines represents hybrid seed that would be used for commercial hybrid seed production.

4. Ensuring Purity of Parental Lines and Hybrids - Approach and Methodology

4.1 On-station and on-farm training

Train farmers and the seed industry to efficiently implement the recommended field isolation distances, spacing, A : B and A : R ratios, rouging, awareness of pollination options, identification of biotic and abiotic constrains and implementation of integrated management practices and planting and harvesting precautions.

4.2 Development and application of molecular purity kits

Molecular markers will be used to identify mixtures of A, B, R, hybrids and common varieties. Nuclear molecular markers will allow differentiating A from R lines and also A and R from other common varieties planted in surrounding areas (Fig 1). Mitochondrial markers (associated with the cytoplasm) will allow differentiating A from B lines containing different cytoplasm, but being iso-nuclear). In-house experiments will be carried out to develop