Euphytica (2019)215:202
https://doi.org/10.1007/s10681-019-2520-4

REVIEW

# Can a speed breeding approach accelerate genetic gain in pigeonpea?

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Received: 12 July 2019 / Accepted: 28 October 2019


**Abstract** Pure line breeding is a resource-intensive activity that takes 10 years or more to develop a new cultivar. In some crops, conducting off-season nurseries has significantly reduced the length of the breeding cycle. This approach could not be exploited in pigeonpea [*Cajanus cajan* (L.) Millsp.], because traditionally it has been a photoperiod-sensitive crop that requires long periods of darkness to induce flowering. However, the recent success of breeding early maturing photoperiod-insensitive genotypes has opened up the possibility of adopting 'speed breeding' techniques to enable rapid generation turnover. This paper outlines a speed breeding approach that integrates the use of immature seed germination for rapid generation advancement and a "single pod descent" method of breeding. To accelerate line development, while conserving genetic variability, the approach permits four generations per year and can fast-track field evaluation of resulting homozygous lines. Therefore, the breeding strategy conserves resources and has potential to deliver new early maturing cultivars within a substantially reduced timeframe of 4–5 years.

**Keywords** Early maturing cultivars · Immature seed germination · Pigeonpea · Rapid generation turnover · Single pod descent method

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## Introduction

Researchers are making all-out efforts to enhance pigeonpea production by (1) inventing new crop rotations, such as pigeonpea-wheat, etc.; (2) adoption of hybrid technology with 30–40% yield advantage; and (3) exploring new production niches in non-traditional areas, such as low and mid-hills and some parts of arid areas. In this context, breeding of high yielding early maturing (90–120 days) cultivars is a priority (Vales et al. 2012; Singh et al. 2016). However, progress is limited by the time it takes for a breeding cycle. For instance, the development of inbred cultivars through pedigree breeding traditionally takes about 10 years, and any reduction in this duration is highly desirable. This review/perspective

Published online: 14 November 2019

highlights the current challenges and opportunity to adopt a new breeding approach that could reduce breeding time of early maturing pigeonpea cultivars by half.

## Background

Fixing useful additive genetic variation in inbred lines is the prime objective of plant breeders. To achieve this, segregating populations are grown in the main cropping season, so that the genetic differences among genotypes would express properly to facilitate single plant/progeny selections. In this approach, the processes of generation advancement and selection are carried forward simultaneously, and therefore, it takes more time and resources to develop a cultivar. To overcome this constraint, Goulden (1941) proposed rapid generation turnover and postponing the selection activity until the breeding populations reach homozygosity. For rapid generation advancement and conserving genetic variability of the cross, the methodology of single seed descent was recommended. This involved harvesting a single seed from each plant of the population, and bulking them together to advance the generation without selection. Brim (1966) called it the "modified pedigree method". Fehr (1991) further modified it and referred to it as "multiple seed descent" or "modified single seed descent" method, where instead of a single seed, 2–4 seeds from each plant are bulked for generation turnover. A similar technique has been developed in soybean, where a single pod is harvested from each plant for generation advance; referred to as the "single pod descent method".

Brim (1966), Mukade et al. (1973), and DePaw and Clarke (1976) have proposed the use of off-season nurseries at sites where the environment is conducive to floral induction and seed setting for generation advancement. In pigeonpea, it is not possible to cultivate traditional pigeonpea germplasm in the off-season due to its photo-period sensitivity. However, since early maturing (90–120 days) genotypes are photo-insensitive (Turnbull et al. 1981; Wallis et al. 1981) and can reproduce in the off-season, accelerated generation turnover appears to be a viable option in breeding early maturing pigeonpea cultivars.

## Speed breeding approach

Recently, speed breeding has been in the spotlight because it provides an ideal platform to accelerate the process of inbred cultivar development (Li et al. 2018). This can significantly reduce the time required for advancing breeding materials from one generation to the next. Rapid plant growth and early flowering can be achieved by optimising the growing environment and/or applying plant hormones. Techniques for rapid generation advancement have been reported in various cereals, legumes and oilseed crops (Watson et al. 2017; Ghosh et al. 2018; Zheng et al. 2013). Among the legume crops, rapid generation turnover has been demonstrated in a few species. In chickpea (*Cicer arietinum* L.), Gaur et al. (2007) demonstrated that three seed-to-seed generations can be achieved within a year. They performed two generations under open field conditions and one under rainout shelters by exposing plants to extended (24 h) photoperiod. By applying plant growth regulators during in vitro flowering and the use of immature seeds, Mobini et al. (2015) reported up to seven generations for faba bean (*Vicia faba* L.) and eight generations for lentil (*Lens culinaris* Medik). They applied cytokinines and auxins to induce early flowering and harvested the immature seeds for generation advancement. By altering plant growing conditions and applying growth regulators, Mobini and Warkentin (2016) achieved up to five generations of field pea (*Pisum sativum* L.) within a year. O'Connor et al. (2013) reported a speed breeding protocol for peanut (*Arachis hypogaea* L.) that used controlled temperature and continuous light to accelerate plant development and fast-track a SSD breeding program. They succeeded in reducing the generation time from 145 to 89 days.

In pigeonpea, information on rapid generation turnover is lacking, and the only reports available are by Saxena (1996) and Saxena et al. (2017). They have used a greenhouse facility with natural light and evaporative air coolers using a 5 hp pump operating @ 2870–2900 rpm and a blower wheel delivering air @ 2980–9330 $m^3$/h. This facility maintained a temperature between 28–32 °C and relative humidity at 50–60%. Under these conditions, it was demonstrated that immature seeds of pigeonpea could be harvested and germinated to reduce the generation time by about 3 weeks; and thus allowed four seed-to-seed generations within a year. To achieve high rates of

germination Saxena et al. (2017) recommended that immature seeds should be harvested about 35 days after flowering (Table 1). At this seed age the germination, when sown in pots, germinated properly with no seedling defect and took time (to germinate) similar to the fully mature seeds. Hence, this technology can be successfully used in pigeonpea breeding programmes to achieve rapid turnover of generations. This presents an opportunity to adopt a speed breeding approach to develop early maturing pigeonpea cultivars in a short timeframe. The salient features of this methodology and its implementation activities are discussed below.

To launch an effective generation turnover programme, applying the right environmental conditions, particularly with respect to temperature, is critical. Under natural conditions, this activity should be confined to those areas where winter and summer seasons are relatively mild. In the areas where cool (< 10 °C) winters and hot (> 40 °C) summers prevail, raising an off-season crop would require a facility with adequate temperature control. Since plants of early maturing genotypes invariably have small canopies, pots of 25-cm diameter can be used. In order to conserve genetic variability, top priority should be given to maintaining a perfect plant stand.

An overview of the activities for implementing a rapid generation turnover programme are summarized in Fig. 1. Start-up breeding activities such as selection of parents and hybridization can be completed in the first cropping season in the field. In the same year, the $F_1$ hybrid plants are grown in a glasshouse. At this stage, the genomics-based hybridity test of each $F_1$ seedling should be undertaken to exclude selfed plants from the generation advancement program; and this can be done easily by using polymorphic markers present in the parents.

The next four generations ($F_2$–$F_5$) can be rapidly advanced in the second year in a glasshouse. The $F_2$ bulk should be sown and a healthy single immature pod should be harvested from each plant, 35 ± 2 days after flowering. To grow the $F_3$ generation, one $F_2$ pod should be placed in a separate pot; and immediately after shelling, all the seeds should be treated with fungicide and sown in the same pot. After 2 weeks, retain only one seedling in each pot and record the flowering dates. Again harvest one pod from each $F_3$ plant 35 ± 2 days after flowering to advance the generation. Repeat this process of seed-to-seed advancement in $F_4$ and $F_5$ generations also. In this way, the breeding population can be advanced by four generations within the second year.

In the third year, the final round of generation turnover ($F_6$) can be conducted in the field. This will allow all the plants to produce maximum number of seeds which further can be used for evaluation purpose. At this stage, the population should be used for marker-based screening for target traits, such as wilt and sterility mosaic diseases, etc. Only selected $F_7$ plants should be advanced for seed multiplication in the off-season in a glasshouse. In the fourth year, $F_8$

**Table 1** Information related to pod age at harvesting and seed germination to raise four seed-to-seed generations within a year. Modified from Saxena (1996) and Saxena et al. (2017)

| Cultivar | Generations per year | Time taken to raise 4 generations/year | First harvest (mean) | | 35-Day harvest (mean) | |
|---|---|---|---|---|---|---|
| | | | Pod age (d) | Germination (%) | Pod age (d) | Germination (%) |
| *Super early maturing group* | | | | | | |
| ICPL 85024 | 4 | 15 June–9 May | 26 | 81.10 | 35 | 100 |
| ICPL 87093 | 4 | 15 June–24 May | 27 | 93.70 | 35 | 100 |
| *Extra early maturing group* | | | | | | |
| ICPL 00004 | 4 | 15 June–11 June | 28 | 91.50 | 35 | 100 |
| ICPL 00151 | 4 | 15 June–17 June | 30 | 98.80 | 35 | 100 |

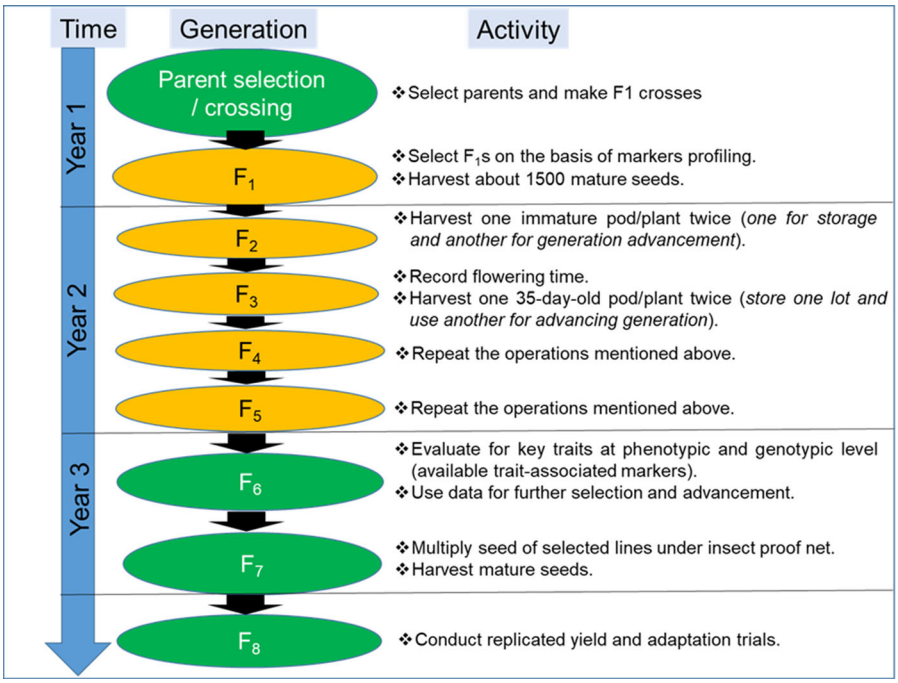


**Fig. 1** An outline for breeding early maturing cultivars using single pod descent method involving accelerated generation turnover and immature seed germination (yellow boxes indicate generations grown in glasshouse and green boxes indicate generations grown in the field). (Color figure online)

progenies can be evaluated in the field along with appropriate controls.

## Discussion

An important feature of the speed breeding technique in pigeonpea is early seed harvest. Therefore, it is important to understand the seed development stages. In general, seed development involves three key phases. Cell division and its expansion takes place in the first week and is governed by auxin signalling (Quint and Gray 2006). In the next 3 weeks, active nutrient accumulation takes place; and this is associated with various energy-generating processes. After about 1 month of development, the seeds start losing moisture to gain maturity. In pigeonpea, information on seed development and its germination is limited. Rao and Rao (1975) reported that dry weight of the developing pigeonpea seeds steadily increased due to continuous deposition of food reserves. Singh et al. (1980) studied various physio-chemical changes in developing seeds and reported that rapid starch accumulation occurred between 14 and 28 days after flowering, while the soluble sugars recorded increases up to 35 days, and then declined slightly. Different amino acids and minerals accumulated to the maximum level in the first 7 days of seed development and hardly changed thereafter. In soybean, the pod wall played an important role in carbohydrate acquisition and created a temporary sink before their remobilization and transfer into the developing seeds (Dubs and Grimes 2000). Balkrishanan et al. (1984) reported no germination in 14-day-old pigeonpea seeds. They recorded 50% seed germination at 21 days, which increased to 70% on the 35th day; and 72–88% at maturity. According to Rao and Rao (1975), the accumulation of food reserves directly influenced seed germination. Singh et al. (1980) suggested that for immature seed germination, seed proteins and amino acids were not important and that the key factor was the accumulation of starch which provided instant energy for germination and initial seedling growth.

Perusal of a different set of flowering and pod-setting data, published earlier by Srivastava et al. (2012) revealed large genetic variation for the time taken from flowering to maturity within extra early maturing pigeonpea genotypes (Table 2). The first

**Table 2** Genotypic differences for seed filling and maturation in six extra early maturing inbred lines. Modified from Srivastava et al. (2012)

| Genotype ID | Time (days) | | | Seeds/pod | 100-Seed weight (g) |
|---|---|---|---|---|---|
| | Flowering | Maturity | Difference | | |
| *Type I progenies* | | | | | |
| 4–8 | 54.00 | 82.00 | 28.00 | 3.70 | 5.90 |
| 5–11 | 56.00 | 89.00 | 33.00 | 3.70 | 6.70 |
| 9–1 | 50.00 | 82.00 | 32.00 | 3.60 | 6.80 |
| Mean | 53.30 | 84.30 | 31.00 | 3.67 | 6.47 |
| *Type II progenies* | | | | | |
| 4–1 | 51.00 | 100 | 49.00 | 2.90 | 8.00 |
| 8–13 | 50.00 | 100 | 50.00 | 3.60 | 8.30 |
| 9–6 | 50.00 | 100 | 47.00 | 3.90 | 6.20 |
| Mean | 50.30 | 100 | 48.60 | 3.47 | 7.50 |

group, on average, took 31 days from flowering to maturity; while in the second group this period was extended by over 2 weeks, to 48.6 days. Such differences may appear due to the presence of different genetic regulatory mechanisms which control photoperiod reaction. Besides controlling floral induction, such genes also induce indeterminateness in pigeonpea plants, which extend their reproductive phase and result in significant delays in pod setting. Hence, keeping in view the genetic nature of this trait, it is advisable that information on the duration from flowering to pod setting also be gathered in parental lines. This will help in scheduling the harvesting of growing pods for generation advance.

The proposed speed breeding methodology has the potential to accelerate the process of breeding early maturing cultivars in pigeonpea. This study offers a possibility of exploiting rapid generation turnover and single pod descent methods in breeding pigeonpea, some new issues may emerge in its implementation. Hence, more research is needed to make this technology suitably efficient at delivering quality products. The benefits of single seed descent method and rapid generation turnover in plant breeding are well documented and, truly, the proposed breeding method is its extension, but with a difference—of forcing immature seeds to germinate for advancing the generations. Apart from this, it also infuses the marker-based selection technology in the breeding program. The proposed scheme, in principle, knits together known breeding technologies such as single seed descent, accelerated generation advance, and marker-based screening to conserve key resources and shortens the time required in breeding cultivars.

Pigeonpea has a large variation for maturity and its germplasm has broadly been classified into early (100–150 days), medium (161–200 days), and late (> 250 days) types. Since pigeonpea is a photosensitive crop, its floral induction takes place at the onset of short days with $\leq$ 10 h of light. According to Silim et al. (2007), warm weather combined with long photoperiods promote a longer vegetative phase, while short photoperiods and milder mean ambient temperatures (> 18 and < 25 °C) promote flowering. They also mentioned that the optimum temperature for rapid flowering in sensitive pigeonpea germplasm is 18.3 °C. Interestingly, early flowering is directly associated with photo-insensitivity (Wallis et al. 1981; Saxena 1981). The association between flowering and photo-sensitivity is so strong that a long duration photo-insensitive cultivars are yet to be developed.

Besides potential advantages of speed breeding technology in pigeonpea, there are certain limitations in its general application in crop breeding. These could be in terms of partial loss of advancing populations which could lead to the erosion of useful genetic variability necessary for single seed descent breeding. Another, limitation could be its limited application to breeding only early maturing pigeonpea cultivars. This is due to the inherent characteristic of photosensitivity and the early genotypes are photo-insensitive, and in contrast the later types have strong short day requirement for floral induction. Further, the use of a fully controlled environment facility for inducing

flowering in late types can be costly for a breeding program. Simulating breeding scenarios that combine rapid generation advance with other technologies like genomic selection (GS), would be a nice strategy to optimise breeding approaches in a cost effective manner (Hickey et al. 2019). Moreover, recent efforts in re-sequencing different pigeonpea germplasm accessions along with high density genotyping have the potential to overcome the issue related to strong linkage between maturity and photo-sensitivity and also provides opportunity to combine GS with the proposed speed breeding approach in pigeonpea. Once the genes/markers associated with photo-insensitivity are identified, then medium and late maturity materials could be advanced through rapid generation turnover.

**Acknowledgements** Authors are thankful to the Department of Agriculture Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Government of India; United States Agency for International Development (USAID); Department of Biotechnology, Government of India; Ministry of Agriculture, Government of Karnataka and ICRISAT for funding various research activities in pigeonpea. This work has been undertaken as part of the CGIAR Research Program on Grain Legumes and Dryland Cereals (GLDC). ICRISAT is a member of CGIAR Consortium.

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