Ethiop. J. Agric. Sci. 31(1) 1-10 (2021)

Rapid Generation Advance in Chickpea for Accelerated Breeding Gain in Ethiopia:
What Speed Breeding Imply?

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Abstract

*Chickpea (***Cicer arietinum** *L.) is grown in a wide range of environments and cropping systems and its maturity ranges from 80 to 180 days.. Time-saving breeding is key to responding to the dynamics of demands and environmental changes. The study employed Single Seed Descent (SSD) technique in advancing the generation,* *supported by an independent observation of chickpea seed germination and seedling establishment in the seed lab. The filial generation nursery was derived from 46 initial crosses with the aim of enhancing drought and yield response of otherwise commercial 10 cultivars. Between 5 December 2017 and 20 December 2018 we were able to obtain four rounds of working chickpea seeds (F_2-F_5) using two research locations. The average time required to obtain early matured pods varied from 80 to 85 days. Harvesting four generations in an annual cycle enables a saving of at least 50% time in variety release, which has the potential to double the rate of genetic gain and variety replacement. As long as measures are taken to reduce risk associated with extreme weather events or animal damage, this low-cost rapid cycling approach could be adapted for large-scale breeding programs to fast track the development of more productive varieties.*

Keywords: Chickpea, early pod generation, programmed stress, speed breeding

Introduction

There is key roles of the crop improvment over decades to enhance the productivity (CSA, 2018), revenue generation power (Setotaw *et al.*, 2018), farming culture (Pachico, 2014) of Chickpea (*Cicer arietinum* L.) in Ethiopia, which is the secondary centere of its diversity (Yadeta and Geletu, 2002). Modern breeding approaches are increasingly being deployed in legumes such as chickpea to enable the rapid development of improved varieties with enhanced yields under challenging climatic conditions (Varshney, 2016). Understanding the genetics of simple or complex traits has involved the use of multiple approaches such as QTL mapping (Asnake 2016b, Varshney *et al.*, 2014), association genetics (Thudi *et al.*, 2014; Varshney *et al.*, 2019). The draft genome sequence and resequence information on germplasm lines (Varshney *et al.*, 2019) has provided opportunities to harness chickpea's whole diversity for trait improvement. Since understanding these traits by developing multi-parent populations is resource intensive and time consuming, the rapid development of populations will enable trait dissection and development of new varieties.

Climate change events along with dynamics of product demands necessitate rapid breeding cycles and swifter replacement of old varieties to ensure speedier genetic gains and sustainable food and nutritional security (Li *et al.*, 2018). The scientific community's endeavors to feed an ever-growing population and bridge the gap between the demand for and supply of nutritious food in a climate change scenario has been a daunting task. It is predicted that feeding a global population exceeding 9 billion by 2050 will require 2% genetic gain (Li *et al.*, 2018). The length of a breeding cycle or generation interval is inversely proportional to the rate of genetic gain. Although over 350 improved varieties of chickpea have been released across the globe, unlike in the major cereals, the development of new varieties and variety replacement rates have been slow in legumes (Gaur *et al.*, 2007).

Most breeding programs evaluate fixed lines which must be generated *via* crossing parental lines and subsequent inbreeding for 5-6 generations. However, crop duration and photoperiod sensitivity hinders the achievement of multiple generations per year. Although chickpea's maturity ranges from 80 to 180 days depending on genotype, soil moisture, temperature, latitude and altitude; most breeding programs routinely achieve two generations per year - one in the field during the crop season and the other in the off-season either in a greenhouse or in an off-season irrigated nursery. While many use double haploid technology to accelerate development of homozygous (inbred) lines (Ren *et al.*, 2017), yet efficient methods are not yet readily available in chickpea. Rapid generation cycling or advance has been used in several crops to accelerate breeding including *in vitro* culturing, embryo rescue or simplified biotron or photons based extended growth techniques. The recent rapid generation advance technology also known as 'speed breeding' uses extended photoperiod and controlled temperature to grow up to 6 generations of chickpea per year (Watson *et al.*, 2018; Ghosh *et al.*, 2018). However, to control the environmental conditions it requires investment in infrastructure, which can be challenging to establish and /or adapt in every breeding program of the less resource system.

As a low-cost and easily adaptable alternative, this study reports a field-based technique for rapid generation advance that can be used to cycle at least four generations of chickpea per year. The methodology providing in chickpea is easy to adopt, simple but effective, and can facilitate fast-track development of populations for pre-breeding and breeding to ultimately enhance the development of improved varieties.

Materials and Methods

This study aims to develop applicable and easily adaptable field-based technique for rapid generation advance to accelerate development of inbred lines. The demonstrated activities formed part of a breeding pipeline to generate elite chickpea materials with favorable combinations of drought tolerance, yield and other agronomic traits. A total of 46 different F1 combinations were generated by crossing 10 released chickpea varieties (Dimtu, Mariye, Teketay, Mastewal, Natoli, Akaki,Teji, Ejere, Acos-Dubie and Chefe) with 10 improved molecular breeding lines (MABC 4, MABC18, ICCMABCA 30, ICCMABCD 19, MABCB 4, MABCB 5, MABCB 3, MABCB 7 and MABCB 2) (Supplementary Tables 1&

2). The released varieties were selected for superior traits of yield, seed size, taste, adaptation, and tolerance to diseases like fusarium. Whereas, the molecular breeding lines /meting parental blocks/ were developed by ICRISAT through marker-assisted selection targeting traits for enhanced drought tolerance.

All generations (F_1 to F_5) were grown in the field following rapid cycle technique newly proposed. The experiments were conducted under rain fed and irrigated conditions at two different field stations: Debre Zeit (8°44' 4.56"N and 39° 0' 30.6"E) and Werer (9°16'N and 40°9'E) agricultural research centers experimental sites in Ethiopia. The F_1, F_3, F_4 and F_5 generations were grown at Debre Zeit and only the F_2 generation was grown at Werer. The experiment was conducted on black clay soils with substantial clay loam. Key management procedures were adopted such as supplementary irrigation as needed, using good soil and good field crop management (weeding, pest protection, hail/ rain protection, wild pet protection, bird protection at planting, excess water management in case of the rainy season) procedures. In the off-season, plants were grown within a fenced plot or mesh-house to protect them from wildlife.

Early Generated Pods: To reduce the length of each generation, an early generated pod (EGP) harvest technique was employed. The aim was to sample /pick/ pods as early as possible while maintaining reasonable germination and plant survival rates. To determine the optimal pod sampling /picking/ time physiological maturity markers was considered, and hence pods from F_2 populations were picked from matured podding zones. The sampled pod seed from each F_2 plant was subjected to germination testing and establishment of progeny in the open growing field condition.

Based on workability of the technique, all proceeding generations were advanced by sampling the most mature pods of the earliest time from the main stem on average about 6 weeks after the onset of first flowers. At this stage, the EGPs appeared yellow or pale in color and contained fully-formed seed (Figure 1). Since chickpea bears its pods sequentially starting from main stems and from the base to tip (acropetally), those earlier set pods are physiological mature earlier (2-3 weeks in advance) compared to pods developed later.

A total of 602 F_2 seeds were harvested from 164 F_1 plants. To rapidly develop inbred lines, a Single Seed Descent (SSD) approach was employed, where a Single-seed from a Single-pod of a Single-plant (SsSpSp) was advanced per generation (F_2 to F_5). EGPs in each generation that were physiologically mature with appropriate moisture content (15-25%) were picked and used to advance each generation, before totally harvesting the plant. The EGPs seeds obtained were re-sown at high density to fasten reproductive processes. At the first pod setting stage, irrigation was stopped (programmed stress) to further hasten pod filling and maturity (Watson *et al.*, 2018).

Results and Discussion

The scientific community's endeavors to feed a growing population and bridge the gap between the demand for and supply of nutritious food in the face of climate change has been a daunting task. If feeding a global population of 9 billion by 2050 require 2% genetic gain in crop improvement programs (Li *et al.*, 2018, Watson *et al.*, 2018), so does feeding an estimated 170 million Ethiopians by the same period is unthinkable without innovative crop improvement approach. With the yield potential of crops reaching a plateau, achieving faster genetic gains is possible only through the introduction of new favorable alleles and rapid breeding cycles (Li *et al.*, 2018). The time it takes to grow a generation is often a limiting factor when it comes to plant breeding because it largely determines the length of the breeding cycle. Keeping all other factors constant (such as selection intensity, genetic diversity and selection accuracy), the length of a breeding cycle is inversely proportional to the rate of genetic gain. Hence, accelerating generation advance to reduce the length of the breeding cycle can lead to enhanced genetic gains in crop improvement programs. Reducing generation time can also benefit pre-breeding and research programs. For instance, accelerating population development in these programs would lead to new insight and tools to support breeding in a shorter timeframe.

This current study successfully demonstrated how to achieve multiple generations per year in the field by exploiting acropetal pod setting and maturation behavior as well as by subjecting the plant to moisture stress at early pod formation stage (programmed stress). This was demonstrated using accelerating line development scheme for materials derived from 46 cross combinations where flowering commences on the main stem and lower branches and proceeds acropetally at intervals averaging 1.5–2 days between successive nodes along each branch. Bulk of chickpea yield emanates from branches stemming from the first three nodes (https://grdc.com.au/__data/assets/pdf_file/ 0022/301639/GRDC-GrowNotes-chickpea-Southern-region.pdf).

Based on pod formation, maturity and their subsequent use for the next generations, the chickpea pods were grouped into five cluster zones (A to E) (supplementary Table 1 and Figure 1). Cluster A represents pods borne by plant that was not subjected to stress and grown under normal conditions. Clusters B, C, D and E represent early, intermediate early, intermediate late and late maturity, respectively. The EGPs were used to advance to the next generation (Figure 1). Average days to flowering ranged between 38 and 45 days after sowing and the early pods generated matured (get ready for picking) between 80 and 85 days in pod cluster B (supplementary Table 1). This allowed the harvesting of four successive generations (F_2-F_5) in one year (2017-2018) under open field under both rain-fed and irrigated conditions.

Table 1: Podding clusters and their usefulness for generation advance

Pod clusters	Pod number on the branch	Maturity status	Moisture level of the seeds (%)	Germination and survival in field (%)	Estimated time saving per generation	Facility required for effective generation advance
A	Dry harvested seed (check)	Physiologically mature and dried seeds	11-12	Outstanding (>95%)	None	Open field
B	1-4	Early pods, mature	15-20	Excellent (>90%)	~3 weeks	Adapted open field
C	5 -8	Intermediate early	18-22	Weak (~50%)	~4 weeks	Manage adaptation, sterilized soil
D	9 -14	Intermediate late	20-23	Weak (~40%)	~5 weeks	Fully controlled environment
E	14- 20	Late pods with late maturity	22-24	Poor (~0-10%)	~6 weeks	Fully controlled environment

Based on the evaluation, standard germination, speed of germination, seedling shoot and root length, seedling vigor index, seedling establishment (b) varied by sources of pod-seed zonation (a) (Figure 1). Accordingly seed germination was fast and establishment was vigor for seed sources obtained from tip of the zonation, however, seedlings were less adaptive to external environment and the vice-versa. Hence, for field based condition *physiologically matured pods* were advisable to apply, as they establish in reasonable duration (4-5 days) and adapt external field growth conditions (supplementary Table 1 and 2). Earlier emergence and establishment of young seeds could be explained by seed growth factors /hormones/ responsible for germination are active at younger than old pods, as in similar way active tip plant tissues regenerate faster than older ones.

We observed a difference of about 2-3 weeks between the formation of the first (^{1}) and last pods (^{20}) pods within a stem. The time between early set of functional pods (physiologically matured) on the stem and complete maturity (fully and completely dried plant ready for harvest) was about 2-3 weeks, making it a total of about 4-6 weeks. One to two weeks' difference in flowering was observed between seeds grown normally and those grown under programed stress (moisture and density). Under normal conditions, 115-125 days were required to harvest functional pods (physiologically matured dried crop) compared to 80-85 days for those grown under programmed stress conditions. This saved an average of 30% of time.

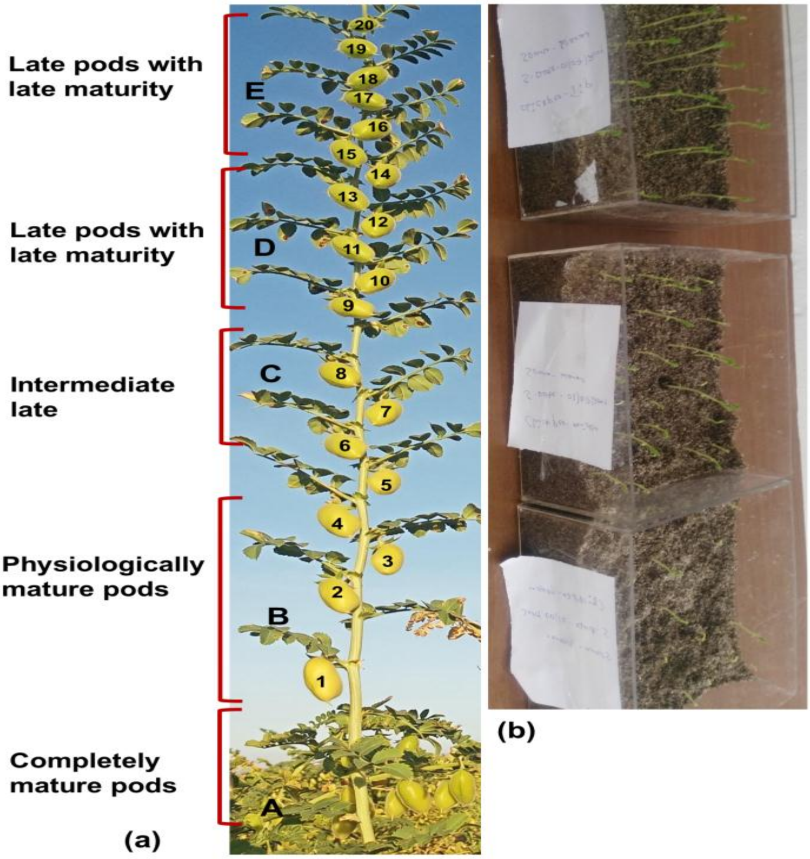

Figure 1: Clusters of pod zonation and developmental processes, which can be exploited for speed breeding in chickpea. Acropetal maturation behavior of the pods (a), Germination speed after 4 days of seeds harvested from different clusters in sterile sand pots (b)

As mentioned above, speed of germination, establishment of seedlings and other seed quality traits varied significantly in different podding clusters (Figure 1b) sources. Although seeds harvested from clusters C, D and E fast germinated in sand pots, their subsequent establishment in the open field could not be realized as seedlings become vulnerable that might need special hardening set-up. Seeds harvested from these clusters would have to require a controlled facility, for enhanced advantage in the area. However, seeds harvested from cluster B germinated well under direct field sown conditions. Previous studies using immature seed germination and SSD method have reported obtaining 3-4 generations per year, for instance in the case of pigeonpea (Saxena *et al.,* 2019). Using speed breeding techniques that control photoperiod/temperature and perform early seed harvest, up to 6 generations per year has been achievable in spring wheat (*Triticum aestivum*), durum wheat (*T. durum*), barley (*Hordeum vulgare*), pea (*Pisum sativum*) and canola (*Brassica napus*) (Watson *et al.,* 2018).

Table 2: Information on cross combinations and F_{2s} used in the study.

Cross	**F_1s**	**F_2s**	**Cross**	**F_1s**	**F_2 s**
Dimtu × ICCMABCA30	3	4	Akaki × MABC 4	2	10
Dimtu × ICCMABCA23	3	12	Akaki × MABC 18	5	14
Dimtu × ICCMABCD19	3	12	Akaki × ICCMABCA30	3	12
Mariye × MABC 4	3	4	Akaki × ICCMABCA23	4	10
Mariye × MABC 18	4	14	Akaki × ICCMABCD19	3	15
Mari × ICCMABCA30	3	10	DZ-10-11 × MABC 4	2	9
Mari × ICCMABCA23	3	16	Teji × ICCMABCB4	3	8
Mari × ICCMABCD19	3	8	Teji × ICCMABCB 3	5	50
Teketaye × MABC 4	4	12	Teji × ICCMABCB 7	4	15
Teketaye × MABC 18	5	10	Teji × ICCMABCB 5	3	16
Teketaye × ICCMABCA30	2	8	Teji × ICCMABCB 2	5	31
Teketaye × ICCMABCA23	3	10	Ejere × ICCMABCB4	10	30
Teketaye × ICCMABCD19	4	14	Ejere × ICCMABCB 3	4	14
Mastewal × MABC 4	4	12	Chefe × ICCMABCB7	3	12
Mastewal × MABC 18	3	10	Chefe × ICCMABCB5	5	14
Mastewal × ICCMABCA30	3	13	Chefe × ICCMABCB 2	4	17
Mastewal × ICCMABCA23	2	8	AcoseDubie × ICCMABCB4	3	15
Mastewal × ICCMABCD19	3	10	AcoseDubie × ICCMABCB 3	5	13
Natoli × MABC 4	4	13	AcoseDubie × ICCMABCB 7	4	16
Natoli × MABC 18	2	10	AcoseDubie × ICCMABCB 5	4	21
Natoli × ICCMABCA30	4	10	AcoseDubie × ICCMABCB 2	2	10

The novel field based accelerated generation cycling approach (Asnake and Tulu, 2019) could improve breeding efficiency and faster delivery of improved chickpea varieties for the farming community. The approach is simple to adopt, effective, and requires low cost investment compared to other rapid generation advance methods that require high cost infrastructure (Watson *et al.,* 2018). A similar approach for field based rapid cycling of rice was developed by the Bangladesh Rice Research Institute, which uses raised/flatbeds that are ideal for managing bigger breeding populations (Rahman *et al.,* 2019). Importantly, the field-based rapid cycling approach in chickpea is best suited for breeding programs located in tropical and sub-tropical regions where weather conditions permit the growth of chickpea all year round. However, as the approach involves growing plant generations in the field, it is critical to adopt risk management practices to protect valuable breeding materials from extreme weather events and wildlife.

Complimentary to the current work, Ethiopia’s diverse ecology provides 30-40% developmental plasticity to chickpea; manipulating this plasticity can further accelerate breeding. For example, based on field score, the days to maturity of commercial chickpea varieties Arerti and Habru differ significantly in the three eco-strategic locations of the warmer lowlands of AlemTena (20-25ºC) station; intermediate of Debre Zeit (18-23ºC) station and cool highland of Chefe-donsa (15-19ºC) stations. Accordingly, analyzing into long term field scoring book of the routine experiments, the days to maturity for Arerti extends from 88 days at AlemTena to113 days at Debre Zeit, and to 151 days at Chefe-donsa locations. Similarly, days to maturity for Habru variety extended from 84 days at AlemTena to110 days at Debre Zeit and to 141 days at Chefe-Donsa locations. Therefore, there is a great opportunity to reduce generation cycle time in chickpea by manipulating through its early-matured pods and/or its phonological class and/or moisture stress treatment and/or high population density and/or growing agro-ecological locations.

Conclusion

Speed breeding is a concept to save time for faster genetic gain and technology development process. There could be different approaches to attain this goal. However, field based acceleration could be taken as a novel approaches in breeding for crops like chickpea with sequential /acropetal or basipetal/ seed maturation behavior. The current information would shade light on chickpea speed breeding using cheap and non-sophisticated techniques.

Acknowledgements

The authors acknowledge the support of the Tropical Legumes (TL) III project in carrying out this study.

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