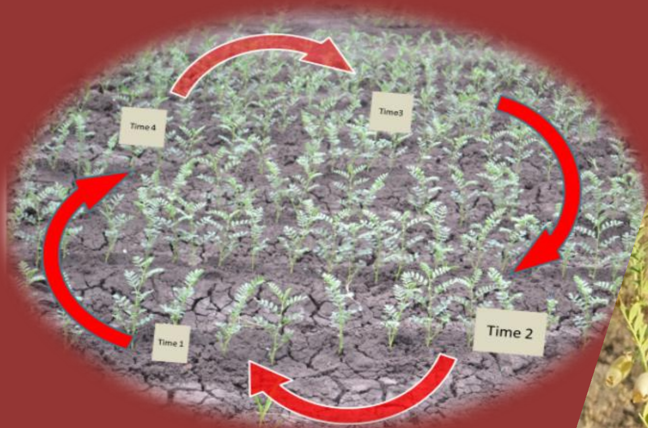
A Guide to Accelerated Breeding Cycle in Chickpea to Enhance Rate of Gain



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ICRISAT
Science with a human face
International Crops Research Institute
for the Semi-Arid Tropics

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Contents

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1. Background

Speed breeding is time saving based approach and is among genetic improvement enhancers approach through genetic recombination and fast generation reconstitution in plant breeding. Genetic gain acceleration enables combating the gap between demand and supply. Dagnachew *et al.,* (2016), have reported a positive yield and seed quality gain over the four decades of Ethiopian chickpea research improvement endeavor. Increasing the number of generations per unit of time (over a given period) improves the overall efficiency in the delivery rate of defined variety or product/s. Amy *et al.,* (2018) have reported that under controlled environment of growth chamber, speed breeding approach achieved up to 6 generations per year for spring wheat (*Triticum aestivum*), durum wheat (*T. durum*), barley (*Hordeum vulgare*), chickpea (*Cicer arietinum*) and pea (*Pisum sativum*), and 4 generations for canola (*Brassica napus*), instead of 2–3 under normal glasshouse conditions.

Under uncontrolled open field condition Asnake *et al* (unpublished) has reported 4 generations per year in chickpea, with possible further potential. It was also noted that the approach can easily be adopted, integrated with other conventional and/or advanced breeding approaches like molecular breeding. This manual tries to elaborate the approaches /which is flexible/ in chickpea speed breeding with the goal to achieve multiple generations per year without losing breeding values (the useful attributes of the products). Single Seed Decent (SSD) Technique is preferred approach along the speeding generation to be made in the course.

2. Importance

The traditional crop improvement rate could not be able to feed the world population where a billion plus of the global population is already facing food and nutritional hunger. IFPRI (2019) reported that many regions of the world faced increasing rates of hunger—with global undernourishment continuing to rise for the third year in a row —and stagnation in tackling malnutrition. A United Nations report found that conflict and climate change were key factors holding back countries' progress in achieving the Sustainable Development Goals (SDGs). Thus, to mitigate the malnutrition and hunger, speed breeding technique could undoubtedly contribute much in a changing climate.

Under traditional breeding approach, in which only one generation per year, or hardly two generations per year are generated, the development of a variety could take not less than a decade. This has consequences on rate of the gains and crop improvement.

In reducing the breeding cycle, thus to improve the rate of product (defined breeding lines, varieties, techniques, etc.) delivery, plant breeders have come up with notable strategies including; *shuttle breeding, double haploid technique, genomic selection, genome editing, speed breeding,* all of which have a common interest (i.e time saving under the citrus paribus principle), keeping all factors similar but time saving. This manual illustrates how chickpea breeding cycle can effectively be reduced to enable fast product/variety delivery for improvement. Key elements of the attributes with speed breeding are:

A. It saves time

B. It realistically enables fast achieve goal

C. It saves resources

D. It enhances genetic gain and rate of variety replacement

E. Improve productivity

3. Multiple Cycle VS Breeding Methods

In reducing breeding cycle, as mentioned above, breeders have used several tools or strategies including shuttle breeding, double haploid techniques, genomic selection, genome editing and speed breeding. In this manual we emphasize on speed breeding in self-pollinated crop of chickpea, with possible spillover to other crop species.

Hence, the current subject deals with speed breeding cycle in chickpea breeding. Furthermore, multiple crop cycle per year is well matched with Single Seed Decent (SSD) advancement scheme. SSD is the classic procedure of having a single seed from each plant, bulking the individual seeds, and planting out the next generation. This approach was initiated since 1940s, from the interest of plant breeders to rapidly inbreed populations before evaluating individual lines.

SSD approach is a particular relevance to exploit the chickpea phenology, that enables fast advancement using Early Emerging Pods (EEP) and programed stress both of which synergize into fast generation achievement per unit of time. The time advantage is F_2-F_5 during selection.

Early Emerging Pods (EEP): Chickpea has acropetal (base to tip) type of pod setting and maturation. Thus, using the EEP can be regarded as gear shifters in speed breeding of chickpea as it can save about 3 weeks for the plant to come into a complete maturity. Seed dormancy in chickpea is not a case at all, thus, physiologically the pods mature from the base to the tip /acropetal type/. Picking a single pod/seed of the matured early emerging ones from the base (Fig 1) in densely populated chickpea to reestablish the next generation is time validated. However, this pattern may be species specific, for instance in tef seed maturation follow a basipetal (tip to base) pattern, where breeders have to follow a reverse direction to chickpea.

Fig 1: EEP emerges on main stem from the base, and it can serve as a base for establishment of the next generation following the SSD scheme

4. Single Seed Decent

The concept is the use of a single seed representing a single plant for generation advance starting from F_2 and can be followed same scheme to F_5. We apply single seed decent or single pod decent right from F_2 to F_5. The model is presented and illustrated in Tables 1 and 2.

In a nutshell, the single seed descent method is the modification of bulk method of breeding. But the modification is in such a way that it allows the equal survival of segregates. The idea of this method was first suggested by Goulden (1941) and subsequently modified by Brim (1960).

General principles involved in this method is that, only one (single) seed collected from each of the F_2 plants (eg. 10000 to 20000) and then bulked to grow the next (F_3) generation. Similar practiced is continued till F_5 or F_6 generation until desired level of homozygosity attained. In F_5 and F_6 generation, when individual's plants are selected and harvested separately, their progenies are also grown separately in the next generation. Selection is done among the promising one to conduct replicated yield trials and quality test conducted in F_7- F_8 generation and coordinated yield trial in F_9-F_{10} generation.

The breeding Procedure in this method is that only one seed is selected randomly from each plant in F_2 and subsequent generations. The selected seed is bulked and is used to grow the new generation. This process is continuing up to F_5 generation. By this time the desired level of homozygosity is achieved. In F_6, large number of single plants, like 200-500 are selected and their progeny is grown separately. In F_7 and F_8, selections are practiced among progeny and superior ones are isolated based on preliminary replicated trial. The superior progenies are then tested in multiplication trials and the best progeny is identified for release.

The main objective of single seed descent method is to rapidly advance the generation of crosses and at the end a random sample of homozygous genotype is obtained.

Among the advantages, in SSD are:

1. Single seed descent method advances the generations with the possible speed in a conventional breeding method,
2. It requires very little space, resource and labours ,
3. It makes the best use of greenhouse and offseason nursery, facilitates because in that multiple generations can be raised in each year,
4. It ensures that the plants retained at the end of population are random sample from F_2 population.

However, with SSD scheme it does not permit any form of selection in natural or artificial and population size varies between successive generation due to poor germination or other cases.

Key processes described as hybridization breeding with one seed from each plant in each generation, then select superior line after F6 for Preliminary yield trial and subsequent coordinated multilocation performance and release processes.

Table 1. Illustration of stepwise in generation establishment and advancing using SSD scheme

Action Steps in SSD Techniques in Self Pollinated Crops			
crossin g		Rmk	
AXB	Crossing parental lines	OO	
F1	Space planting, harvest in bulk	Selfed	
F2	Densely planted, one random seed picked per plant and bulked *Each color group of F_2 represents a single plant with bunch of pods*	OOOOOOOOOO OOOOOOOO	A C C E L E R A T E
F3	>>	OOOOO	
F4	>>	OOOOO	
F5	>>	OOOOO	
F6	Space planted and 100-500 plants with desirable x-stics harvested separately	OOOOO	
F7	Individual plant progenies grown, desirable & homozygous progenies harvested in bulk; Target adaptation for target test		
F8	PYT with suitable check and target adaptation test=-		
F9	Coordinated yield trail; Disease, quality, adaptation test		
F10	Variety Verification Test and release		

Table 2 : Hypothetical schematics of possible population development and advancement in chickpea breeding

Master-board in Breeding Development Scheme (SSD)			
double	single	#	Area
AxB=E & CxD=F			Note: speedy cycle can be done from F2 to F5, which takes like 2 yrs if 4 generation is effected
ExF	AxB=	50 successful crosses	[determined by level of resources and facility and experience]
F1	F1	50 plants space planted ---full maturity (FM)	10cmx20cm
.	F2	50 plant X 20 seed=1000 densely planted –partial maturity(PM)	10x15
.	F3	1000- densely planted -PM	10x15
.	F4	900- densely planted -PM	10x15
.	F5	800- densely planted -PM	10x15
.	F6	700- space planted -FM	10x20 harvested for progeny establishment, seed source,
.	F7	600- progeny row planted and selection/discard-FM	10x20 one row each progeny on target env't [10%-20% SI)
.	F8	PYT (in sets if needed)-FM	~ 60 to 120 lines in 2-3 mkt product trait groups 4-6 location performance evaluation
.	F9	Multilocation yield trail= 40-60 pro lines - FM	4-6 divergent location performance evaluation
.	F10	VVT/release 2-5 varieties space planted-FM	
.	F11	seed increase	

5. Procedures in Field Level Operation

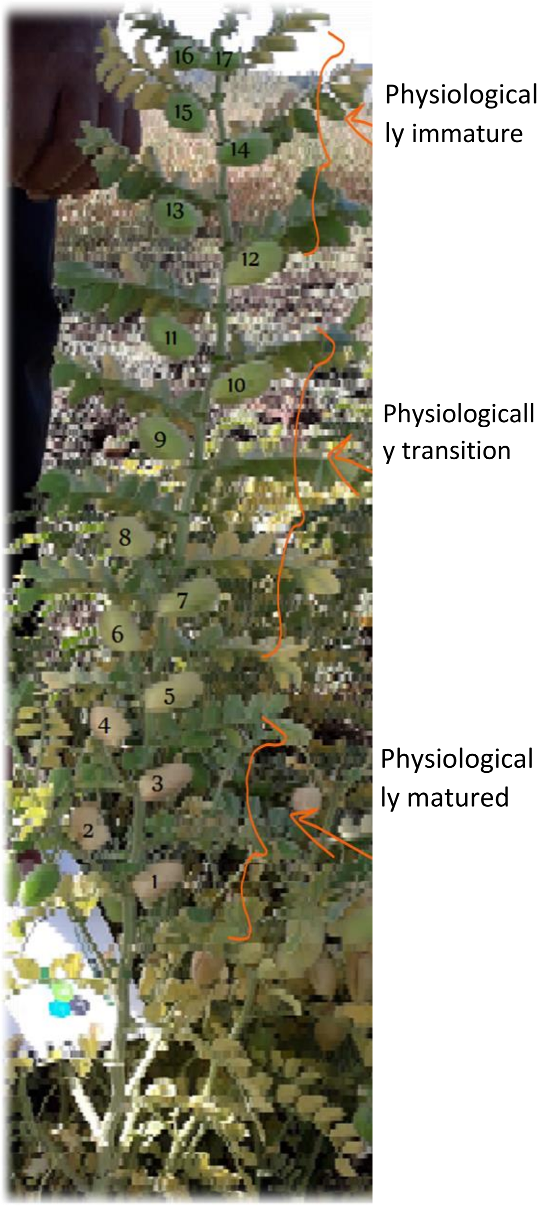
We employ our crossing blocks like other recurrent set up in chickpea. Once identification of parental lines is sorted purposely, plots for establishing the resources would be made. Crossing processes would be effected based on the trait of interests for introgression. The crossing design could be unidirectional (one only as male and the other as female) or reciprocate as need be. The crossing program could have different clusters of crossing blocks for underlying objects. Ones F1 are generated it would be advanced to F2. At F2 all seeds from F1 harvest would be planted and at poding single seeds would be picked to reconstitute F3 and the same procedure can be applied till F5. This segregating period help application of SSD scheme in attaining acceleration.

If the materials are early or late phenology groups, it influences the time to be saved and product delivery rate to some extent. Under ideal circumstances where year-round plant growth is a possibility, realistically one can propose, variety release speed can be reduced at least by half maintaining the difference among phenology groups. This could be attained in average climatic environments like in

Debre Zeit, where the climatic extremes are low. We still advise speeding is a multitude of innovative option explored and to be explored than fixed term.

Time saver interventions in chickpea (can be used in separate or combination)

1. **<u>Acropetal EEP Advancement</u>**: Chickpea pods grows from base to tip /acropetal/. Using Early Emerging pod (EEP) as next generation advancement, the difference between the first and last pod with in a bearing main stem takes about **2-3** weeks' time gap advantage.
2. **<u>Physiological Maturity Vs Dry crop</u>**: using physiological maturity (when pods turned yellow) saves time, hence, the time between early set pods (Fig. 1) and field dried



harvest ready crop is about **4-6** weeks.

3. **<u>Programed Stress Induction (PSI):</u>** PSI is an intentional stress induction procedure through moisture stress at booting or high population density stress (2-3x the normal) or by extended light environment (16-18hrs); all of which pose crop phenological responses by phenological signaling action. It saves 1-3 weeks difference in phenological processes between normally grown and the ones grown under programed stress
4. Special media techniques to grow young green pods (this is not covered in this tech. manual)

Fig. 1 Acropetal maturation of pods/seeds in chickpea, give opportunity for fast advancement using SSD.

6. Time Saving Value

The time saving advantage in SSD scheme on average is about 30-35% to make a complete cycle of the crops or generation. For instance, it was possible to harvest functional physiologically matured pods in about 80-85 days while under full maturity dry harvest condition it takes 115-125 days for intermediate maturity groups.

Table 2: designing ideal time rate in days in breeding cycle among different phenology groups in chickpea

SSD breeding scheme in chickpea under year round growth condition														
phenology group	crossing	F_1 [50:50]	$F_{2\ SSD}$ [75:25]	$F_{3\ SSD}$ [87.5:12.5]	$F_{4\ SSD}$ [93.75:6.25]	$F_{5\ SSD}$ [96.88:3:12]	$F_{6\ bulk + obs}$ [98.44:1.56]	$F_{7\ perfo.\ Eva}$	$F_{8\ perf.\ Eva}$	$F_{9\ vvt\ and\ release}$	Gapping days	total days	yrs
LM ± 5	130	130	90	90	90	90	130	130	130	130	100	1240	3.44
IM ± 5	115	115	80	80	80	80	115	115	115	115	100	1110	3.08
EM ± 5	100	100	70	70	70	70	100	100	100	100	100	980	2.72
SEM ± 5	85	85	55	55	55	55	85	85	85	85	100	830	2.31
Traditional one season per yr												4380	12
Traditional 2 crop/year												2190	6
							Bulk, observation, line cluster setting (sharing to partners)	Phenoty + evalua for major traits	Phenoty + evalua for economic traits	vvt			

**The 100 Gapping days refers to the number of days for preparations/adjustments in between every plantation; L-I-E-SE= M refers late, intermediate, early and super early maturity groups with estimated 5 days variability.*

7. Managing Population Number

The population size with SSD is expected to decrease to some degree with each generation advancement (due to lack of germination, lack of seed set, stress etc) starting from maximum at F2. Hence, there has to be a plan considering what number is targeted. In the reality of expected decrease with subsequent generation, for example if one targets 200 F4 plants and 70% of the seeds in each generation will produce plants with at least one seed. Then, by cascading back to the F_2 generation, one need to consider establishing 584 F_2 plants to work with. A hypothetical graph demonstrating the population number in progress is depicted in Figure 2 below.

Demo Note: The speed breeding piloting experience in chickpea execution of generation advancement improvement in 2017/18 have shown an than 517 days between parental lines planting date and harvest date of F_5, using two locations of Were and Debre Zeit. Incidence of devastating ascochyta on F_2 could pose a dramatic decrease in the number of progeny lines. Hence, the current manual took into consideration potential encounters.

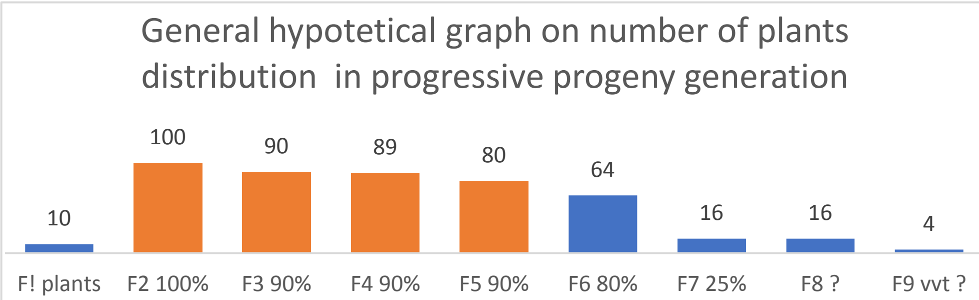


Figure 2: General generation advancement trend (possible but not fixed)

Trial locations: Trial locations could be one or multiple (shuttle) based on deriving facility and climatic factors. However, a lot of factors come in the play pre-determining this. Under contained environment where all factors can be controlled a location could be enough. Even under environments where the annual seasonal variability or climatic extremes are low like Debre Zeit, a a location can serve the purpose. However, if seasonal variability in climatic factors is high to the level it interferes with crop/flower development, one may need more additional locations with suitable conditions in the targeted period. Example; the different climatic conditions among the different agroecology gives opportunity to use like shuttle scheme. However, it is important as much possible to avoid any environmental condition that deter flower development and fertilization effectiveness.

Planting media: Progeny lines can be planted on plots, pots or trays under conditioned or normal environments. The soil media could be in no difference than the normal chickpea common growing soil. All crop management practices (seed treatment, crop management, plant protection etc) can be applied accordingly.

Summary of activities

- *Develop crossing design /select parents, trait of interest/*
- *Harvest F_1 and advance to F_2 and allow full plant maturity; at F_2 this stage genotyping of young plant leaves DNA for tracing markers of trait of interest can be superimposed*
- *Plant all F_2 harvest seeds*
- *Pick a single pod/seed from each plant to reconstitute F_3 using SSD*
- *Do same as above till F_5*
- *Select and harvest plants at F_6 with desirable characteristics, and reject inferior qualities*
- *Further select and constitute lines of F_7 and evaluate performances over locations and stress spots*
- *Do same as above at F_8*
- *Do same as above at F_9 (optional)*
- *Select super-performing lines/s for variety candidate to be evaluated under verification*
- *Go with micro-seed increase of candidates*

8. Reference

Amy Watson, Sreya Ghosh, Matthew J. Williams, William S. Cuddy, James Simmonds, María-Dolores Rey, M. Asyraf Md Hatta, Alison Hinchliffe, Andrew Steed, Daniel Reynolds, Nikolai M. Adamski, Andy Breakspear, Andrey Korolev, Tracey Rayner, Laura E. Dixon, Adnan Riaz, William Martin, Merrill Ryan, David Edwards, Jacqueline Batley, Harsh Raman, Jeremy Carter, Christian Rogers, Claire Domoney, Graham Moore, Wendy Harwood, Paul Nicholson, Mark J. Dieters Ian H. DeLacy, Ji Zhou, Cristobal Uauy, Scott A. Boden, Robert F. Park, Brande B. H. Wulff and Lee T. Hickey. 2017. Speed breeding is a powerful tool to accelerate crop research and breeding. NATURE PLANTS www.nature.com/natureplants.

Dagnachew Bekele, Asnake Fikre, Million Eshete, Nigusie Girma. 2016..Genetic progresses achieved in Ethiopian chickpea (Cicer arietinum L.) breeding program based on grain yield and seed size. In Lijalem Korbu, Tebikew Damite and Asnake Fikre (eds). Harnessing Chickpea Value Chain for Nitrition Security and Commercialization of Smallhoder Agriculture in Africa. Jan 30-2 Feb, Debre Zeit , Ethiopia.

International Food Policy Research Institute. 2019. 2019 Global Food Policy Report. Washington, DC: International Food Policy Research Institute. https://doi.org/10.2499/9780896293502

9. Appendix

I. Characterization of different components

cluster	Phenographic pod cluster	Determined as	Moisture level %	Ave. speed of germination on pure sand box	Adaptation/ survival on open field	Time saving per cycle compared to full time	Remarks
A	Dry harvested seed (check)	Dried seed	11-12	Normal: 7-8 days	full	Full time	Open field
B	1,2,3,4	Early pods, physiological mature	15-20	Late: 6 days	Excellent >90%	~ 3 weeks	Adapt open field
C	5,6,7,8	Intermediate early	18-22	Intermediate early: 5 days	Weak ~50%	~4 weeks	Manage adaptation, sterilized soil
D	9,10,11,12	Intermediate late	20-23	Intermediate late: 5 days	Weak ~40%	~5 weeks	Need special growth envt
E	13,14,15,16, 17	Late pods, late maturity	22-24	Earlier: 4 days	Poor ~ 0-10%	~6 weeks	Need Special growth media envt

II. Comparison of the different approaches on speeding generation

Parameters	Improvised conventional	Australian photon base	Molecular	DH
facility demand	Very low	high	Very high	Very high
Early homozygosity fixing	No before F6+	No before F6+	yes	Yes yes
Technical complexity	low	low	high	high
cost	Very low	high	Very high	Very high
adapted easily	yes	yes	?	?
Out put effect	Effective	Effective	Effective	Effective
Disadvantage	Inferiority may come forward	Inferiority may come forward	High cost	Laborious, less productive and less recombinatio n