

Rapid generation advancement through speed breeding in lentil (*Lens culinaris* Medik.)

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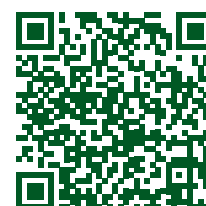
Abstract: Speed breeding (SB) is an effective solution to enhance genetic gain. We advanced 14 intraspecific and interspecific lentil populations from F_2 to F_6 generation under a SB growth chamber applying an extended photoperiod of 18h light (23-25 °C)/6h darkness (14-16 °C). Six generations per year were achieved with an average generation cycle ranging from 62 to 76 days, demonstrating the effectiveness of SB in reducing generation time. More than 1500 F_{6-7} single plant-row advanced lines obtained from the populations were grown as observation nurseries under field conditions for seed multiplication and preliminary selection prior to their introduction in yield trials in the perspective of identifying new improved varieties. In addition to higher genetic gain, the SB method is resource efficient and easy to implement in small-scale breeding programs. To our knowledge, this is the first report showing effective implementation of SB protocol in lentil breeding pipeline in Africa and the Middle East.

Keywords: Speed breeding, extended photoperiod, generation, lentil populations, genetic gain

INTRODUCTION

Lentil (*Lens culinaris* Medik.), a valuable and nutritionally important legume, has been a nutritional staple food for millennia (Zohary et al. 2012). It has continued for centuries, feeding populations around the world through its nourishing seeds rich in iron, zinc, and proteins. Worldwide research in genetic improvement has aimed to enhance disease resistance, increase yields, and improve nutritional quality. In this ongoing research, genetic gain has emerged as the guide in efforts to develop lentil varieties capable of meeting the dynamic demands of modern agriculture and nutrition.

Lentil breeding programs have sought innovative approaches to develop high-performance varieties. However, conventional methods of lentil breeding are characterized by long timelines (Idrissi 2020); many years of intensive effort are usually required to achieve near-homozygosity in advanced lines. This time-consuming process is particularly evident in temperate climates, where a single generation in the field and one to two additional generations under controlled conditions are the annual norm (Roy et al. 2023). Furthermore, the risk of losing genetic material in advancing generations under field conditions as a consequence of biotic and a biotic stress, especially through frequent drought and heat stress, is another challenging aspect and limiting factor of conventional breeding methods.



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Researchers can use speed breeding techniques as alternatives to conventional breeding schemes. This involves growing segregating populations derived from targeted crosses under controlled conditions of light, temperature, humidity, and other parameters, thus considerably shortening generation cycles (Chaudhary and Sandhu 2024). The beginning of this fundamental work (Wellensiek 1962) opened the way to a cascade of innovative strategies aimed at shortening reproductive cycles over a wide range of cultivated species, such as soybean (*Glycine max*) (Jähne et al. 2020), by producing 5 generations a year using an extended photoperiod; chickpea (*Cicer arietinum*) (Watson et al. 2018, Samineni et al. 2020), by producing 6-7 generations a year using an extended photoperiod and immature seeds; cowpea (*Vigna unguiculata*) (Edet and Ishii 2022), by producing 7-8 generations a year using light intensity and immature pods; faba bean (*Vicia faba*) (Mobini et al. 2015), by producing 7 generations a year using embryo rescue of immature seeds, an extended photoperiod, and plant growth regulators, such as Flurprimidol; groundnut (Ochatt et al. 2002), by producing 4 generations a year using embryo rescue of immature seeds, artificial lighting, and plant growth regulators, such as Flurprimidol; and lentil (*Lens culinaris*) (Idrissi 2020), by producing 3-4 generations a year using an extended photoperiod.

Speed breeding is an innovative approach in the field of plant breeding, using simple advancement techniques to accelerate the selection process. The guiding concept is accelerating plant life cycles in order to rapidly generate and create new varieties (Watson et al. 2018). This accelerated process takes place in a controlled environment, where environmental factors such as light exposure, temperature, humidity, plant nutrition, and other parameters are controlled (Mobini and Warkentin 2016, Mitache et al. 2023, Mitache et al. 2024). By manipulating these elements, researchers can create optimal conditions for healthy and rapid plant development, leading to rapid flowering and maturing, thus accelerating the rate at which multiple generations of plants can be produced (Idrissi 2020).

Our main objective is to harness the power of speed breeding to rapidly create climate-resistant lentil varieties. This research paper offers an example of implementation of speed breeding methods for rapid development of $F_{6,7}$ fixed lines for introduction into the lentil breeding pipeline. This simple methodology promises to transform the genetic improvement of intraspecific and interspecific and wild introgression lentil populations, helping to accelerate development of new adapted varieties potentially carrying adaptive alleles from the wild gene pool that may help to enhance the resilience of this important crop for sustainable farming and food security.

MATERIAL AND METHODS

Plant material

Fourteen interspecific (*Lens culinaris* Medik. × *Lens orientalis* Boiss.) and intraspecific (*Lens culinaris* Medik. × *Lens culinaris* Medik.) populations were obtained from simple crosses using 18 accessions as parents. They were selected from the crossing blocks of the National Institute for Agricultural Research of Morocco (INRA, regional center of Settat) breeding program, based on specific desired traits. These accessions were obtained from a lentil breeding program, the gene bank of the INRA, and the International Center for Agricultural Research in Dry Areas (ICARDA) Rabat-Morocco (Table 1).

Photo-thermal and plant growth conditions

Crosses were made in the greenhouse, and the F_1 seeds obtained were then grown as single plants to produce a large number of F_2 seeds to begin the F_2 self-pollinated populations for subsequent advancement of generations within a speed breeding growth chamber. The selected populations were grown in a growth chamber under controlled conditions and an extended photoperiod of 18 hours of light at temperatures between 23 and 25 °C, followed by 6 hours of darkness at temperatures ranging from 14 to 16 °C (Mitache et al. 2023). 'APOLLO 8' broadband lamps (410-730 nm) with an output of 240 W and a light intensity of 74-93 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were used as a light source (Figure 1). The seeds were sown at high density (≥ 150 single plants/0.5 m²) in special crates with a 55-cm length, 35-cm width, and 17-cm height, using a soil:peat ratio of 2:1. Two advancement methods were applied: single seed descent (SSD) for six populations, bulk breeding (BULK) for five populations, and the combination of SSD and BULK for three populations (Table 2). The populations were watered every 4-7 days depending on their growth stage and their water needs.

Table 1. Description of lentil plant material used in crosses

Species	Genotype ID	Use	Traits of interest
<i>Lens culinaris</i>	2005-S-100-3 (JSH)	1	Large seed size, yellow cotyledon, early maturity
<i>Lens culinaris</i>	010S96131-2	1	Erect growth habit
<i>Lens culinaris</i>	2007-S-96803-2	1	Erect growth habit
<i>Lens culinaris</i>	2009 S 96 511-3	1	Erect growth habit
<i>Lens culinaris</i>	2009 S 96 574-3	1	Erect growth habit
<i>Lens culinaris</i>	2017-S-38016-514	1	Erect growth habit
<i>Lens culinaris</i>	2585-ILWL90-4-3	1	Drought tolerance
<i>Lens culinaris</i>	7978ILWL 118-5-2	1	Drought tolerance
<i>Lens culinaris</i>	ABDA	2	Large seed size, yellow cotyledon, semi-erect growth habit
<i>Lens culinaris</i>	Extra	5	Medium seed size, yellow cotyledon, early maturity
<i>Lens culinaris</i>	ILL 6415	1	Orobanche tolerance
<i>Lens culinaris</i>	ILL 7201 x ILL8	1	Erect growth habit
<i>Lens culinaris</i>	ILL 7723	2	Orobanche tolerance
<i>Lens culinaris</i>	ILL6002 x ILL 7978-2	1	Drought tolerance
<i>Lens culinaris</i>	LIRL-21-187	2	Orobanche tolerance
<i>Lens orientalis</i>	LO615670	3	Drought tolerance
<i>Lens culinaris</i>	RIL-115	3	Erect growth habit
<i>Lens culinaris</i>	V5 (08114-1x1056-2)	1	High grain yield, multiple pods per peduncle

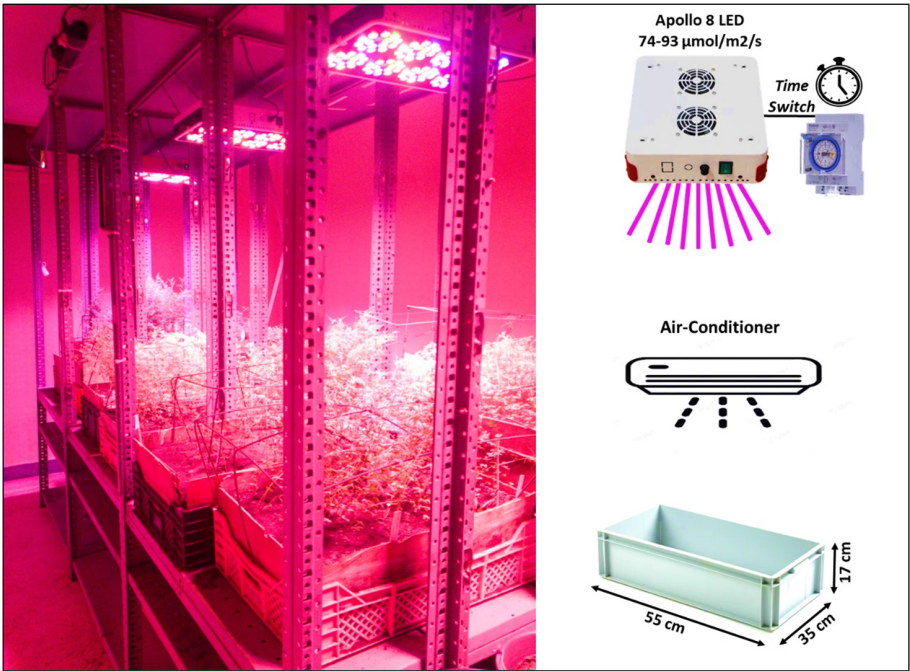


Figure 1. Speed breeding growth chamber and its equipment.

RESULTS AND DISCUSSION

Significant differences were obtained, first for the size of the starting populations (F_2), ranging from 51 seeds for Pop9 to 339 for Pop10 (Table 2), and secondly for the size of the end population (F_6), which reached 2100 seeds for Pop1 using the BULK method. Differences in the size of the starting populations are directly linked to the number of F_2 seeds obtained from single self-pollinated F_1 plants. The larger the number of F_2 seeds from F_1 plants, the larger the starting population size.

Table 2. Description of populations obtained in this study

Code	TC	SPS (seeds)	EPS (seeds)	Population	AM	SBC	AGT (days)	NG
Pop1	Interspecific	70	2100	(♀) 010S96131-2 × (♂) LO615670	BULK	F ₃ – F ₆	76	5
Pop2	Intraspecific	50	28	(♀) 2005-S-100-3 × (♂) F 2003-7	SSD	F ₂ – F ₅	66	6
Pop3	Intraspecific	90	4	(♀) 2007-S-96803-2 × (♂) LIRL-21-187	SSD	F ₄ – F ₆	62	6
Pop4	Wild Introgression	213	44	(♀) 2009 S 96 511-3 × (♂) 7978ILWL 118-5-2	SSD	F ₂ – F ₅	74	5
Pop5	Intraspecific	198	22	(♀) 2009 S 96574-3 × (♂) LIRL-21-187	SSD	F ₂ – F ₅	72	5
Pop6	Intraspecific	70	33	(♀) EXTRA × (♂) RIL-115	SSD - BULK	F ₂ – F ₆	63	6
Pop7	Intraspecific	195	129	(♀) EXTRA × (♂) RIL-115	SSD	F ₂ – F ₃	65	6
Pop8	Intraspecific	53	45	(♀) EXTRA × (♂) V5 (08114-1.1056-2)	BULK	F ₂ – F ₅	66	6
Pop9	Intraspecific	51	1100	(♀) ILL 6415 × (♂) ILL 7723	BULK	F ₂ – F ₅	72	5
Pop10	Intraspecific	339	52	(♀) ILL 7201. ILL8 × (♂) 2017-S-38016-514	SSD - BULK	F ₂ – F ₆	65	6
Pop11	Intraspecific	135	334	(♀) ILL 6002. ILL 7978-2 × (♂) EXTRA	SSD - BULK	F ₂ – F ₄	69	5
Pop12	Wild Introgression	112	440	(♀) ILL 7723 × (♂) 2585-ILWL90-4-3	BULK	F ₃ – F ₄	70	5
Pop13	Back-cross	164	9	BC2 (♀) ABDA × (♂) LO615670	SSD	F ₂ – F ₆	67	5
Pop14	Back-cross	87	420	BC3 (♀) ABDA × (♂) LO615670	BULK	F ₂ – F ₄	90	4

Pop: population, TC: type of cross, SPS: starting population size, EPS: end population size, AM: advancement method, SBC: speed breeding cycle, AGT: average generation time, NG: number of generations, SSD: single seed descent, BULK: bulk-breeding, SB: speed breeding, ♀: female, and ♂: male

The type of advancement method applied affects the size of the end population, whether the single seed descent (SSD) method or BULK method is used, and it also affects the plant mortality rate during advancement of generations under speed breeding conditions. When using the SSD method, there is usually a decrease in population size from F₂ to F₆, because of plant mortality, high competition between plants as a consequence of high plant density, and extended exposure to light that may result in plant injury and stress, such as for Pop2, Pop4, Pop5, and Pop7 (Table 2). In the same way O'Connor et al. (2013) showed that the number of seeds decreased from F₂ to F₃, which was related to plant mortality and emergence rate, pod maturity, seed viability, and the seed recovery rate during advancement of generations. In contrast, when using the Bulk method, the population size tends to increase, as for Pop1 and Pop9. However, even with the Bulk method, the population size may decrease during cycles from F₂ to F₆, due to a high plant mortality rate, such as for Pop8 (Table 2). The remarkable acceleration of generation turnover achieved by speed breeding has enabled us to rapidly evaluate F₆₋₇ accessions in field experiments (Figure 2), facilitating identification of promising, climate-resistant lentil populations with desirable characteristics.

The protocol presented by O'Connor et al. (2013) using speed breeding and SSD methods on peanut (*Arachis hypogaea*) achieved 3 generations per year. However, using the protocol described in this study, six of the selected populations were able to achieve more than six generations a year, seven of the selected populations were able to achieve more than five generations a year, and one population was able to achieve more than four generations a year, in contrast with only one generation under normal conditions (Table 2).

The average generation time ranged from 62 days for Pop3 to 90 days for Pop14 (Table 2), highlighting the role of efficient time management in accelerating generation turnover, an essential factor in speeding up the selection process and optimizing genetic gain. The plant life cycle time is the most important parameter in breeding cycles, which can be reduced by using an extended photoperiod in speed breeding (Watson et al. 2018). In this study, we used a variety of populations, namely interspecific,



Figure 2. Example of single plant-row fixed F₆₋₇ accessions, obtained from speed breeding, grown under field conditions for seed multiplication and preliminary observation.

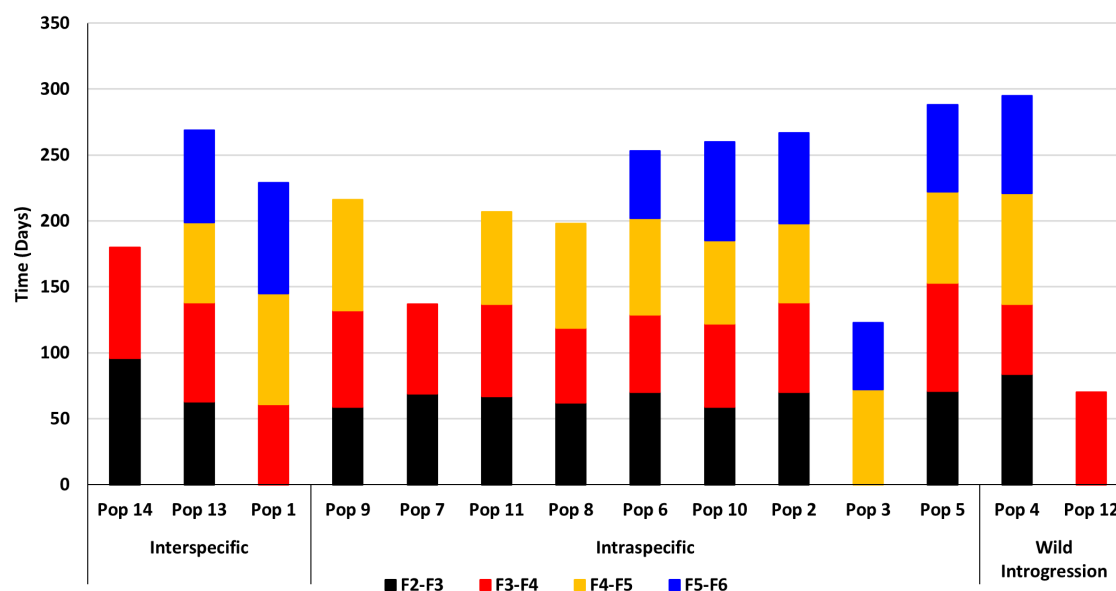


Figure 3. Lentil life cycle length from the (F_2) to (F_6) generations in number of days for each population.

intraspecific, and wild introgression populations. This strategic combination has enriched lentil genetic diversity in the breeding pipeline, potentially joining new targeted traits to develop high-performing varieties. The key element of our strategy was the deployment of different advancement techniques, especially the SSD method. This method has enabled rapid generation of quasi-homozygous lines, thereby speeding up the selection cycle (Wanga et al. 2021) and optimizing genetic gain. In particular, for some populations, we integrated the SSD method with the BULK method, enabling faster progress and maintaining higher genetic diversity (Jana and Khangura 1986) within the segregating populations. We believe that combining these two methods could help to achieve higher genetic gain while maintaining a higher number of fixed lines that will proceed to the next steps of breeding pipelines. This will enrich the diversity of the breeding line set to be screened and may enhance the likelihood of identifying candidate varieties carrying the targeted traits.

The results obtained in this study revealed important temporal variations among populations (Pop1 to Pop14), advancement methods (SSD, BULK, and SSD-BULK), generations (F_2 to F_6), and types of crosses (interspecific, intraspecific, and wild introgression) (Figure 3). These variations can be attributed to the morphology and phenology of the parents used in the crosses. Our results are in agreement with those of Lulsdorf and Banniza (2018), which showed differences in days to maturity among lentil generations and populations. Significant differences in generation time compared with conventional selection methods were also observed by Ghosh et al. (2018) and Samantara et al. (2022). Use of the SSD method with speed breeding can be resource efficient, as described by O'Connor et al. (2013).

The results obtained confirm the added value of speed breeding based on application of an extended photoperiod and advancement techniques in lentil breeding. These innovative approaches can considerably reduce the time needed to develop improved varieties. The results of this study demonstrate not only the scientific value of this method, but also the practical benefits it offers. The protocol used here, known for its simplicity, efficiency, and cost-effectiveness, once again indicates that it can be extensively adopted in lentil breeding programs.

CONCLUSION

In this study, we investigated the effectiveness of combining a speed breeding technique, based on an extended photoperiod, with various advancement methods to increase lentil genetic gain. Our research was based on a diversity of populations, including interspecific and intraspecific crosses and wild introgression populations. The single-seed descent (SSD) method played an important role in rapidly obtaining quasi-homozygous lines, which can help speed

up the selection cycle and maximize genetic gain. Speed breeding has enabled some populations to advance more than six generations per year, compared with a single generation under normal growing conditions in a greenhouse. Our approach may open the way to exploring optimization of genetic gain in other crops. Future research could focus on early selection methods based on genomics within the perspective of enhancing the frequency of desired traits in the final fixed F_6 populations to be introduced in the advanced steps of the breeding pipeline. This study confirms the importance of speed breeding and advancement methods to accelerate the development of improved lentil varieties, offering new opportunities to meet the growing needs of agriculture and for food.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

REFERENCES

- Chaudhary N and Sandhu R (2024) A comprehensive review on speed breeding methods and applications. **Euphytica** **220**: 42.
- Edet OU and Ishii T (2022) Cowpea speed breeding using regulated growth chamber conditions and seeds of oven-dried immature pods potentially accommodates eight generations per year. **Plant Methods** **18**: 106.
- Ghosh S, Watson A, Gonzalez-Navarro OE, Ramirez-Gonzalez RH, Yanes L, Mendoza-Suárez M, Simmonds J, Wells R, Rayner T and Green P (2018) Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. **Nature Protocols** **13**: 2944-2963.
- Idrissi O (2020) Application of extended photoperiod in lentil: Towards accelerated genetic gain in breeding for rapid improved variety development. **Moroccan Journal of Agricultural Sciences** **1**: 1.
- Jähne F, Hahn V, Würschum T and Leiser WL (2020) Speed breeding short-day crops by LED-controlled light schemes. **Theoretical and Applied Genetics** **133**: 2335-2342.
- Jana S and Khangura BS (1986) Conservation of diversity in bulk populations of barley (*Hordeum vulgare* L.). **Euphytica** **35**: 761-776.
- Lulsdorf MM and Banniza S (2018) Rapid generation cycling of an F_2 population derived from a cross between *Lens culinaris* Medik. and *Lens ervoides* (Brign.) Grande after aphanomyces root rot selection. **Plant Breeding** **137**: 486-491.
- Mitache M, Baidani A, Bencharki B and Idrissi O (2024) Exploring the impact of light intensity under speed breeding conditions on the development and growth of lentil and chickpea. **Plant Methods** **20**: 30.
- Mitache M, Baidani A, Houasli C, Khouakhi K, Bencharki B and Idrissi O (2023) Optimization of light/dark cycle in an extended photoperiod-based speed breeding protocol for grain legumes. **Plant Breeding** **142**: 463-476.
- Mobini SH and Warkentin TD (2016) A simple and efficient method of in vivo rapid generation technology in pea (*Pisum sativum* L.). **In Vitro Cellular & Developmental Biology - Plant** **52**: 530-536.
- Mobini SH, Lulsdorf M, Warkentin TD and Vandenberg A (2015) Plant growth regulators improve in vitro flowering and rapid generation advancement in lentil and faba bean. **In Vitro Cellular & Developmental Biology-Plant** **51**: 71-79.
- O'Connor DJ, Wright GC, Dieters MJ, George DL, Hunter MN, Tatnell JR and Fleischfresser DB (2013) Development and application of speed breeding technologies in a commercial peanut breeding program. **Peanut Science** **40**: 107-114.
- Ochatt S, Sangwan R, Marget P, Yves Placide A, Rancillac M and Perney P (2002) New approaches towards the shortening of generation cycles for faster breeding of protein legumes. **Plant Breeding** **121**: 436-440.
- Roy A, Sahu PK, Das C, Bhattacharyya S, Raina A and Mondal S (2023) Conventional and new-breeding technologies for improving disease resistance in lentil (*Lens culinaris* Medik). **Frontiers in Plant Science** **13**: 1001682.
- Samantara K, Bohra A, Mohapatra SR, Prihatini R, Asibe F, Singh L, Reyes VP, Tiwari A, Maurya AK, Croser JS, Wani SH, Siddique KHM and Varshney RK (2022) Breeding more crops in less time: A perspective on speed breeding. **Biology** **11**: 275.
- Samineni S, Sen M, Sajja SB and Gaur PM (2020) Rapid generation advance (RGA) in chickpea to produce up to seven generations per year and enable speed breeding. **The Crop Journal** **8**: 164-169.
- Wanga MA, Shimelis H, Mashilo J and Laing MD (2021) Opportunities and challenges of speed breeding: A review. **Plant Breeding** **140**: 185-194.
- Watson A, Ghosh S, Williams MJ, Cuddy WS, Simmonds J, Rey MD, Asyraf Md Hatta M, Hinchliffe A, Steed A and Reynolds D (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. **Nature Plants** **4**: 23-29.
- Wellensiek SJ (1962) Shortening the breeding-cycle. **Euphytica** **11**: 5-10.
- Zohary D, Hopf M and Weiss E (2012) **Domestication of plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin**. Oxford University Press, 264p.