Euphytica (2020)216:178
https://doi.org/10.1007/s10681-020-02715-6

Speed breeding in pea (*Pisum sativum* L.), an efficient and simple system to accelerate breeding programs

Federico Cazzola · Carolina Julieta Bermejo · Maria Fernanda Guindon · Enrique Cointry

Received: 27 February 2020 / Accepted: 11 October 2020

Abstract Time in breeding programs is a key factor. Shortening plant cycles allows to increase the efficiency of the programs. The objective of this work is to compare different rapid generation technologies on commercial varieties and then apply the most efficient on two segregating populations in order to develop a simple and low cost speed breeding system in pea. Three methods were evaluated. One completely in vitro that gave very poor results. An in vitro–in vivo system, which shortened the varieties cycles with an intermediate efficiency, and an in vivo method that also shortened the cycles and was selected for its greater efficiency (51–95%) and lower cost. It consisted in a hydroponic system, with a 22-h photoperiod supplied by fluorescent T5 tubes, a temperature of 20 ± 2 °C, flurprimidol antigiberelin and early grain harvest. This method applied to segregating populations presented higher efficiencies than the traditional SSD in the field achieving up to five generations per year. This system called Speed Breeding, includes a simple hydroponic system in a growth chamber, with controlled temperature and photoperiod, flurpimidol antigiberelin and anticipated grain harvesting. Does not require a high investment and allowed to increase the program efficiency significantly, reducing the necessary space (266 plants/m^2), the costs and labor.

Keywords Pea · Speed breeding · Rapid generation · Flurprimidol · In vivo method

F. Cazzola (✉) · C. J. Bermejo · M. F. Guindon · E. Cointry
Cátedra de Mejoramiento Vegetal y Producción de Semillas, Facultad de Cs. Agrarias, UNR, Zavalla, Santa Fe, Argentina
e-mail: cazzola.f@gmail.com

F. Cazzola · C. J. Bermejo · M. F. Guindon · E. Cointry
IICAR-CONICET, Instituto de Investigaciones en Ciencias Agrarias de Rosario, Campo Experimental Villarino, Zavalla, Santa Fe, Argentina

Introduction

Pea (*Pisum sativum* L.) is a major cool-season pulse crop and an essential component of sustainable cropping systems (Duc et al. 2010; Jensen et al. 2012). In 2017, dry pea represented the third most important pulse crop production after common bean and chickpea with 16.20 Mt produced worldwide (FAOSTAT 2017).

Plant breeding based in conventional methods is a slow process. In fact, developing new varieties of crops such as pea needs a decade or more, using traditional methodologies. The method of single seed descent was born out of a need to speed up the breeding program by rapidly inbreeding a population prior to beginning individual plant selection and evaluation, while reducing a loss of genotypes during the segregating generations. The method allows the

Published online: 22 October 2020

breeder to advance the maximum number of F_2 plants through the F_5 generation. This is achieved by advancing one randomly selected seed per plant through the early segregating stages (Goulden 1941; Saxena et al. 2019). The focus on the early stages of the procedure is on attaining homozygosity as rapidly as possible, without selection allowing the maximum expression of additive variance.

An attempt is being made to accelerate the process because it is a limiting factor to obtain improved varieties and in the development of recombinant inbred lines (Ril's). Different alternatives were proposed for the reduction of this period. Brummer et al. (2011) and Atlin et al. (2017) suggested off-season sowing. However, in a crop such as pea, Ochatt and Sangwan (2010) determined that only two generations per year could be obtained by changing the hemisphere or three with the use of greenhouse, although this implies a higher cost to the improvement program. Off-season nurseries (spring–summer sowing) in the same hemisphere is not a reliable alternative due to the significant loss of segregating material because the effect of high temperatures causes flowers, pods and grains abortion (Sita et al. 2017). The scientific community and the companies linked to the breeding, develop continually alternatives that allow a greater efficiency and genetic gain in the breeding programs. Double haploids technology has been one methodology developed, however, the legumes have been described as recalcitrant to this approach (Croser et al. 2006, Germáná 2011) so its implementation is not feasible in addition to being costly for the required equipment while reducing recombination possibilities (Liu et al. 2016).

A second alternative is to use of in vitro culture methodology. In peas, different authors (Ochatt et al. 2002; Surma et al. 2013; Ribalta et al. 2017) have developed it in order to induce in vitro flowering, shortening this period using inductive photoperiods and in some cases applications of hormones. Other authors have used in vitro culture to rescue embryos in various species with the aim of shortening the flowering to maturity period. Gebologlu et al. (2011), in tomato, analyzed the rescue of embryos in different stages using different culture media, Dağüstü et al. (2010), evaluated, in sunflower, the rescue of embryos 10 days after flowering. On the other hand, Bermejo et al. (2016) in lentil and Barroso et al. (2019) in pepper and green pepper, compared embryo culture in MS medium with the conventional system and the best time to extract immature embryos to obtain a complete development of plants. It should be taken into account that in vitro plant regeneration is not 100% efficient and changes in different species (Greenway et al. 2012) and is usually genotype dependent (De la Fuente et al. 2013). The rescue of pea embryos can also be carried out in vivo along with the flowering acceleration using a hydroponic system with photo and thermo periodic control and reducing the growth of plants by applying antigibberelin (Mobini and Warkentin 2016).

According to Chahal and Gosal (2002), the high cost of materials, labor and the need for specialized staff are limiting factors for the incorporation of in vitro acceleration techniques in conventional improvement programs, therefore new technologies that improve the efficiency and decrease costs are demanded (De la Fuente et al. 2013; Varshney et al. 2019; Hickey et al. 2019). The new technologies to develop do not include transgenesis or gene editing due to political, social and economic issues (Eriksson et al. 2019).

In recent years, a system called Speed Breeding was developed in different crops. It includes growth plants in chambers or greenhouses, using artificial lighting with inductive photoperiods, temperature and humidity control and anticipated grain harvest. Different protocols were developed for different species such as peanuts (O'Connor et al. 2013), rice (Collard et al. 2017), barley and wheat (Watson et al. 2018), soybeans (Nagatoshi and Fujita 2018), chickpea (Samineni et al. 2019) and lentil (Idrissi et al. 2019, Idrissi 2020).

At present, there are no papers comparing different methodologies in order to determine their efficiency in accelerated generations and there are no research work using in vivo methodologies in pea without the use of embryo rescue.

The objective of this work is to compare different rapid generation technologies and then apply the most efficient on two segregating populations in order to develop a simple and low cost Speed breeding system in pea.

Materials and methods

The experiments were carried out at the Faculty of Agricultural Sciences of the National University of

Rosario, Zavalla, Santa Fe (33°1′ S and 60°53′ W). Three alternatives of acceleration in pea generations were evaluated: in vitro, in vitro–in vivo and in vivo methodologies.

In all the experiments, varieties belonging to the active collection of the research group were used. From these data, the methodology that best adapted to the objectives of the work was selected and then, the evaluations of two segregating F_2 populations were carried out.

In vitro method

Four semi-leafless commercial varieties were evaluated (Kaspa, Navarro, Amarilla and Turf). Three different explants were sown (complete seed, seedlings with the apex removed and only the apex). After 7 days, germinated embryos will be used as a source of explants. Apical meristems of stems (1 cm in length and comprising two internodes) was extracted from the plants with a scalpel. The plants with the meristem removed and the apical meristems of split stems were used as explants. Both explantes and complete seed were grown in 30 cm long culture tubes containing 20 ml of MS culture medium (Murashige and Skoog 1962) modified with macro and micronutrients and vitamins of B5 medium (Gamborg et al. 1968), 3% (m/v) of sucrose and 0.6% (m/v) of agar–agar. The medium was adjusted to pH 5.6 prior to addition of agar and autoclaved at 121 C for 20 min. As a gibberellin synthesis inhibitor, 0.6 µM of Flurprimidol (α- (1-Methylethyl) -α- [4- (trifluoromethoxy) phenyl] -5-pyrimidinemethanol) was used to control the height of the plant which was incorporated together to the culture medium. A tester without flurprimidol was also used. Ten explant per treatment and 2 replications were performed with a completely randomized design. All culture tubes were exposed to a photoperiod of 20 h of light supplied by fluorescent tubes (T5) (500 µM m^{-2} s^{-1} light intensity) and a temperature of 20 ± 2 °C. Flowering was tagged when the petals exceeded the level of the sepals, when the anthesis is performed (Ribalta et al. 2017). The number of flowers produced by each explant and each concentration of flurprimidol, the days of sowing to flowering and the production of pods by plants were recorded.

In vitro–in vivo method

As experimental material, 3 semi-leafless commercial varieties (Kaspa, Amarilla, Turf) and one normal leaf type (Zavalla 15) were used. A simplified hydroponic culture system was used in a growth chamber with a photoperiod of 20 h of light supplied by fluorescent tubes (T5) (500 µM m^{-2} s^{-1} light intensity) and a temperature of 20 ± 2° C. The seeds of each variety were sown in germination trays (30 slots each and 1 seed per slot) with perlite as a substrate. Hydroponic solution with 6 macronutrients and 11 micronutrients, (Green and Red solution, Verde al Cubo, Buenos Aires, Argentina) was applied 1 or 2 times per week depending on the development of the plants. A randomized complete design with two replications was used. A solution of 0.6 µM of Flurprimidol was applied as an inhibitor of gibberellins synthesis on the hydroponic solution when the seedlings had 3 true leaves. The days to flowering and full cycle (days to seed harvest) were evaluated and the flowers were tagged at the anthesis time. The efficiency of the method considered as the percentage of plants with pods over the amount of plants sown and the height of the plants at the time of harvest were evaluated. The pods obtained were harvested 18 days after flowering, superficially sterilized by immersion in 70% ethanol (v/v) for 5 s., 10 min in 3.5% sodium hypochlorite (v/v) and rinsed 4 times in sterile distilled water. Under aseptic conditions the immature seeds were extracted from the pods, the seminal teguments were removed and grown in two germination media, MS medium (described previously in the in vitro assay) and on perlite. 30 embryos per variety and substrate, in a complete random design with 2 replications were used. The number of germinated embryos per substrate and variety were evaluated.

To compare this methodology, the same commercial varieties were sown in the field, in plots with four rows of 2 m long with 70 cm between rows and 10 cm between plants arranged in a complete randomized design with two replications with a total of 80 plants per plot. The soil was prepared with conventional tillage and the seeds were treated with seed cure (fludioxonil (4- (2,2-difluoro-1,3-benzodioxol-4-yl) -1H-pyrrole- 3-carbonitrile) and metalaxyl-M (N- (2,6-dimethylphenyl) -N- (2′-methoxyacetyl) -D-alanine methyl ester). A drip irrigation system and herbicide Linuron (3- (3,4-dichlorophenyl) -1-methoxy-1-

methylurea) in pre-sowing and Imazetapir (5-ethyl-2-(4-isopropyl-4–methyl-5-oxo. -2 -imidazolin 2 il) nicotinic) as post-emergent to avoid competition with weeds were applied. The plant height before harvest, the days to flowering, the days to harvest and the number of plants harvested on the number of sowing plants were analyzed.

In vivo method

Two semi-leafless varieties (Turf and Amarilla) and two normal-leaf varieties (Ilca 5115 and Zavalla 15) were sown in the same hydroponic system described in the previous method. Thirty individuals per variety, in a completely random design with 2 replications were used. The pods were harvested 24 days after flowering when the grains reached physiological maturity (Ribalta et al. 2017). They were stored on trays and once harvested all the pods were sown to start the next generation. The plant height before harvest, the days to flowering, the days to harvest and the efficiency of the method measured as the number of plants harvested over the total number of plants sown were recorded.

Like the previous method, the same varieties were sown in the field using the planting framework described above and analyzing the same variables.

Segregating Population

After the most efficient method was selected, 90 individuals of a F_2 population from the cross of varieties with green cotyledon (Turf x Ilca 5115) and 90 from an F_2 derived from the hybridization of varieties with yellow cotyledon (Zavalla 15 × Amarilla) were seeded. Each F_2 population was generated from the cross between semi-leafless and normal-leaf varieties, therefore the F_2 population had 75% of plants with normal leafs. They were conducted and evaluated during two recombination cycles in a completely randomized design.

Simultaneously, the same F_2 populations were conducted in the Experimental Field following a Single Seed Descent (SSD) scheme, in horticultural section belonging to the Faculty of Agricultural Sciences of the National University of Rosario, Zavalla, Santa Fe (33° 1′ S and 60° 53′ W). The same soil treatments mentioned above were used. Two rows of 15 m long with 150 plants were sown per population with 10 cm between plants. The traits analyzed were the same of in vitro–in vivo methodology.

Statistical analysis

Normal distribution of the morphological data evaluated in different methods was verified by a Shapiro–Wilk test (Shapiro and Wilk 1965). Data from parents and segregant generations were subjected to analysis of variance (ANOVA) using the statistical software Infostat (Balzarini et al. 2008).

Results

The analysis of the in vitro method did not have the expected results since only two plants reached the flowering period using the apex removed, one from cultivar Kaspa and the other from Turf (Table 1 and Fig. 1). The rest of the plants did not grow enough, produced calluses and/or grew to the top of the culture tube and showed no signs of flowering after 90 days. The use of 0.6 µM flurprimidol reduced the plant height reaching only 20 cm.

In vitro–in vivo method

The data obtained from this method and the data from the field controls are shown in Tables 2, 3 and 4.

The analysis of variance (Table 2) showed that the decrease in height was highly significant ($F = 54.11$; $p < 0.001$) for this trait. The days to flowering as well as the days to full cycle, had considerable decreases with respect to the varieties sown in the field. These differences were also highly significant ($F = 34.9$; $p < 0.001$). The same trend was observed in the full-cycle days ($F = 98.3$; $p < 0.001$). Table 3 shows that the application of flurprimidol produced a considerable reduction in plant height from 51% for the Kaspa variety to 69% for the Zavalla 15.

With regard to efficiency, both methods have similar values except for the Zavalla 15 variety that showed an efficiency of 49%, which is considered low. Taking into account that this is a normal leaf variety and its handling is difficult in the in vitro-in vivo methodology, it would demonstrate a low adaptation of this variety type (Figs. 2 and 3).

Table 1 In vitro method. Percentage of flowering plants from varieties in different explants using 0.6 μM flurprimidol and the control without flurprimidol

	Complete seed		Plant with ápex removed		Apex	
Variety	No Flur	0.6 μM Flur	No Flur	0.6 μM Flur	No Flur	0.6 μM Flur
Kaspa	0	0	0	10	0	0
Navarro	0	0	0	0	0	0
Amarilla	0	0	0	0	0	0
Turf	0	0	0	10	0	0

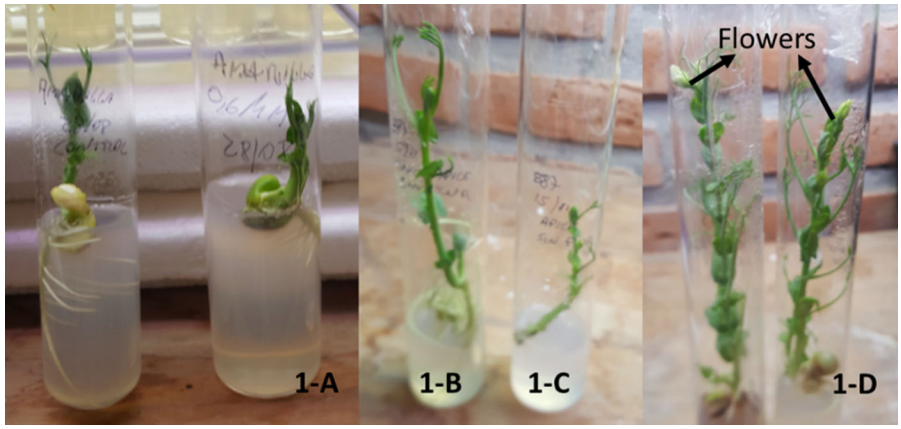

Fig. 1 In vitro method with different pea explant. **a** Complete seed **b** seedlings with the apex removed **c** apex explant **d** Plant with flowers

Table 2 Comparison of methodologies through analysis of variance for Plant Height, Days to flowering and Days to full cycle (days to seed harvest)

		Plant Height		Days to flowering		Days to full cycle	
Comparison methods	DF	MS	F	MS	F	MS	F
In vitro-in vivo versus Control	1	6400	54.11*	6890	34.9*	13,340	98.3*
Error	1	118.27		197.42		135.7	

F F-test

*Significant difference at 5% probability. $p < 0.05$

In reference to the embryo rescue, the substrates evaluated were highly efficient for generating seedlings from 18 DAP embryos, however the use of the MS medium had the disadvantage of producing some seedlings with a reduced radical development, others with callus developments. With this methodology, about 4 generations per year could be carried out.

In vivo method

The dates of analysis of variance and the values for different evaluated traits are presented in Table 5 and 6.

This method showed greater efficiencies in obtaining pods when it was evaluated on the different varieties, following the same trend that was previously mentioned in the in vitro–in vivo method, where semi-

Table 3 Plant height, days to flowering, days to full cycle (days to seed harvest) and the efficiency of in vivo–in vitro method and its field control

	In vivo–in vitro method				Field method			
	Kaspa	Amarilla	Zav 15	Turf	Kaspa	Amarilla	Zav 15	Turf
Plant height (cm)	28[a]	29[a]	35[a]	25[a]	57[b]	51[b]	112[b]	58[b]
Days to flowering	67[a]	65[a]	78[a]	71[a]	97[b]	91[b]	98[b]	93[b]
Days to full cycle	85[a]	83[a]	96[a]	89[a]	149[b]	142[b]	152[b]	145[b]
Efficiency (%)	79	76	49	71	77	78	67	75

Different letter indicate significant difference between both methods at 5% probability $p < 0.05$

Table 4 Percentage of embryos germinated by substrate and variety

Variety	MS medium (%)	Perlite (%)
Kaspa	100	100
Amarilla	100	95
Zavalla 15	100	100
Turf	60	100

leafless varieties (93 and 95%) showed greater efficiency over normal leaf varieties (51 and 58%). The plant height varied between 24 cm and 27 cm (Turf and Amarilla respectively) in the semi-leafless varieties and between 36 cm and 38 cm in the normal varieties (Zavalla 15 and Ilca 5115), presenting a considerable reduction when compared with field materials that had values between 51 cm and 112 cm. These differences were highly significant (F = 31.8;

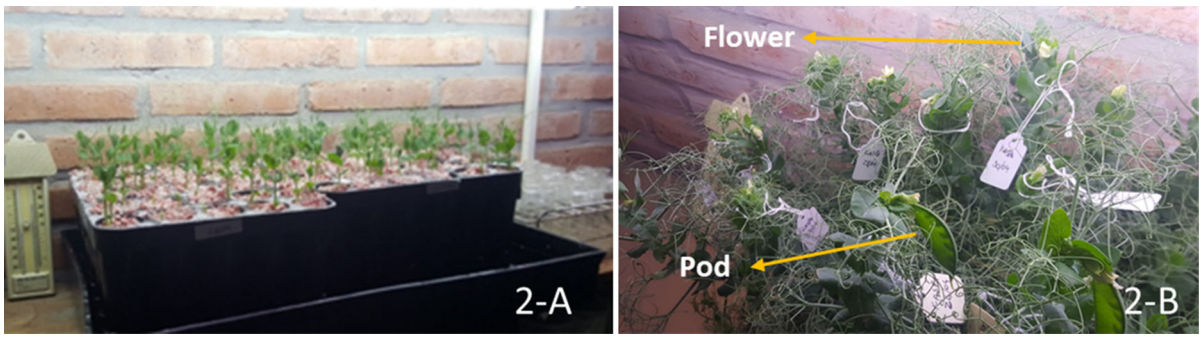

Fig. 2 **a** Simplified hydroponic system for in vivo and in vivo–in vitro methods, using perlite as substrate and hydroponic solution in tray. **b** Flowers and pods in the in vivo system

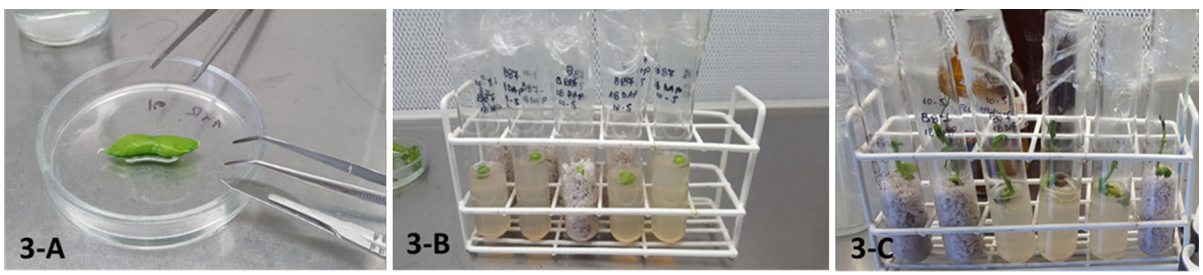

Fig. 3 **a** Rescue of embryos at 18 days after flowering. **b** Embryos culture in perlite and MS medium. **c** Embryos growth

Table 5 Comparison of methodologies through analysis of variance for plant height, days to flowering and days to full cycle (days to seed harvest)

		Plant Height		Days to flowering		Days to full cycle	
Comparison methods	DF	MS	F	MS	F	MS	F
Methods	1	8930.25	31.8*	11,354.2	92.1*	20,592.25	680.74*
Error	1	280.82		123.28		30.25	

F F-test

*Significant difference at 5% probability. $p < 0.05$

Table 6 Plant height (cm), days to flowering, days to full cycle and the efficiency (%) for in vivo methods and their control in the field for the different varieties

	In vivo method				Field method			
	Turf	Amarilla	Zavalla 15	Ilca 5115	Turf	Amarilla	Zavalla 15	Ilca 5115
Plant height (cm)	24^a	27^a	36^a	38^a	58^b	51^b	112^b	93b
Days to flowering	48^a	46^a	57^a	55^a	93^b	91^b	98^b	95^b
Days to full cycle	72^a	70^a	81^a	79^a	145^b	142^b	152^b	148^b
Efficiency (%)	93	95	51	58	75	78	67	68

Different letter indicate significant difference between both methods at 5% probability $p < 0.05$

$p < 0.001$). Semi-leafless varieties had a reduction of approximately 52% in plant height while in normal leaf varieties the reduction was greater, being approximately 63%.

The days to flowering of the materials in the in vivo method varied between 46 days for Amarilla and 57 days for Zavalla 15, while these two materials evaluated in the field required 91 and 98 days to flowering respectively (F = 92.1; $P < 0.001$). The efficiency of the semi-leafless materials was higher than the normal leaf varieties in both methods. However, in the in vivo method the efficiency of the normal leaf varieties was lower than in the field. This fact demonstrates the poor adaptation of this type of materials to the in vivo method.

Comparing the efficiency of the three methodologies and the lower cost required show that the in vivo method is the most feasible to be incorporated into an improvement program.

Segregating populations F_2 and F_3

The plant height was reduced with values around 30 cm for each population and generation while at field had higher values. These differences were highly significant (F = 84.7; $P < 0.001$). The complete cycle in this system, calculating from the sowing to the harvesting of pods, ranged between 65 and 71 days in both population and generations, being lower than in the field (F = 68.5; $P < 0.001$) allowing the realization of 5 generations per year. The efficiency of the in vivo system varied between 74% and 78% being higher than in the field. These values were similar to the average of the efficiency of the parental varieties (Table 6). The seeds obtained by this method were multiplied in the greenhouse and at present, are being evaluated in the field as potential commercial varieties.

Discussion and Conclusions

Due to the long time required by conventional breeding methods in the self-pollinated species for the development of new commercial varieties, it has encouraged scientists and breeders to find faster ways to obtain pure lines (Lui et al. 2016). The modification of the environment where plants are developed, such

as temperature and photoperiod allow a shortening in the development cycles, which is helpful to accelerate the pea improvement programs because are the main factors in the transition to flowering in legumes (Nelson et al. 2010). Actually, there are different protocols to accelerate plant breeding in legumes, for example, in chickpea (Samineni et al. 2019), lentil (Lulsdorf and Banniza 2018, Idrissi et al. 2019) and pigneon pea (Saxena et al. 2019).

In this paper, three different systems developed to accelerate pea generations were evaluated. With respect to the completely in vitro method, Franklin et al. (2000) and Ochatt et al. (2002) proposed two protocols. Both protocols were developed for a limited number of early flowering cultivars and none of them mentions the efficiency of the method. The knowledge of the efficiency is important if the aim of the method is to be used in segregating populations to obtain new Ril's. Following an SSD scheme requires the preservation of the material through the generations of recombination.

Ribalta et al. (2014) reported a method of in vitro flowering using MS medium and Flurprimidol to control plant size. Despite reporting for the full cycle an average of 50 days in short and long cycle varieties, with an efficiency between 70 and 90%, in some long cycle cultivars only 10% of the plants flowered after 90 days of culture. In the present work this method did not show encouraging results since very few plants with flower formation were obtained, it is also a method with a high cost and that must be carried out by trained researchers. Due to these characteristics, it is not a feasible technology to be incorporated into breeding programs.

The in vitro–in vivo method includes an in vitro stage in which immature embryos are cultivated on culture media in order to shorten the plant cycle and then includes an in vivo period where the plant ends its development. It has been tested on peas (Surma et al. 2013) as well as on lentil (Bermejo et al. 2016). Ribalta et al. (2017) evaluated the efficiency of in vitro rescue of embryos at different stages, demonstrating that their extraction at 18 DAP (days after pollination) was superior. In our case, the extraction at 18 DAP (in vitro–in vivo method) resulted in an average cycle of 88 days for the varieties evaluated (Table 2). The in vitro-in vivo methodology compared with the field showed very promising results with respect to the duration of the cycle. However, the cost involved in the in vitro technique is considerable due to the requirements of specific equipment and specialized labor (Ghosh et al. 2018). On the other hand the harvest of the pods at physiological maturity (24 DAP) using the in vivo method showed a duration of 76 days (Tables 5 and 6), which is beneficial because the rescue embryo and the use of aseptic conditions are not necessary. The prolongation of the cycle when the embryos were extracted at 18 DAP was due to the slow development of the plants during the first days of acclimatization. Similar results were reported by Surma et al. (2013), who observed a very slow growth during the first 3 weeks. The plants flowering between 1.5 and 2.5 months after in vitro planting and a 10% loss of the plants during acclimatization.

The completely in vivo method using a simplified hydroponic system was the best in relation to the efficiency of the system to preserve variability and also simpler and more economical, becoming the most feasible to be incorporated into a breeding program. This system allowed in pea to obtain between 4.5 and 5.2 generations per year (Tables 7 and 8). On the other hand, the conventional method in the field allowed us only one generation per year (Tables 7 and 8). Mobini and Warkentin (2016) had already proposed an acceleration system of generations entirely in vivo using the rescue of immature embryos at 18 DAP and cultivating them on perlite as a substrate which decreased the cost of the culture media. However, the method requires extra work with the disadvantage that the germination of embryos is irregular, which does not allow the proper management of segregating generations. The harvest of the seeds at 24 DAP (in vivo) allows to save the seed, enabling, unlike the other methods, the pause between generations improving the system.

In all the systems, flurprimidol and fluorescent tubes were used to grow the plants.

The Flurprimidol was used to produce compact and smaller plants that facilitated the work in the breeding chamber. This hormone block cytochrome P450-dependent mono-oxygenases, which catalyzes the oxidation of ent-kaurene in ent-kaurenoic acid, therefore, inhibits the biosynthesis of gibberelic acid (Rademacher 2000). When inhibiting gibberellic acid, elongation of internodes does not occur, consequently decreasing the height of the plants. In the present experiment and in previous works (Mobini and Warkentin 2016; Ribalta et al. 2014), Flurprimidol

Table 7 Comparison of methodologies through analysis of variance for plant height, days to flowering and days to full cycle (days to seed harvest)

Comparison methods	DF	Plant height		Days to flowering		Days to full cycle	
		MS	F	MS	F	MS	F
Methods	1	3205	84.7*	6885	48.1*	11,325	68.5*
Error	1	37.83		143.13		165.3	

F F-test

*Significant difference at 5% probability. $p < 0.05$

Table 8 Average plant height at harvest (cm), days to flowering and full cycle and efficiency of the method (%) and its field control in segregating populations

In vivo method						Field method		
	F_2 Green	F_2 Yellow	F_3 Green	F_3 Yellow	F_2 Green	F_2 Yellow	F_3 Green	F_3 Yellow
Plant height (cm)	33^a	31^a	30^a	32^a	68^b	82^b	62^b	74^b
Days to flowering	45^a	46^a	41^a	47^a	96^b	94^b	90^b	92^b
Days to full cycle	69^a	70^a	65^a	71^a	148^b	145^b	140^b	143^b
Efficiency (%)	78	76	75	74	61	64	68	71

Different letter indicate significant difference between both methods at 5% probability $p < 0.05$

reduced the size of pea plants, which is important specifically in tall genotypes and for use in cropping systems in confined spaces.

Mobini and Warkentin (2016) comparing the use of three different light systems (T5 fluorescent, LEDs, and high-pressure sodium plus metal-halide lamps) did not show significant differences in days to flowering in pea. However, the latest advances in LED lighting and control of the environment in greenhouse as in growth chambers allow to be a technology that can be incorporated into modern improvement programs (Watson et al. 2018; Idrissi et al. 2019). However, these lighting technologies still require an initial investment greater than other conventional ones such as fluorescent tubes.

The segregating populations conducted through SSD scheme in the field had an efficiency of 66% (Tables 7 and 8). These values are in concordance with Knauft et al. (1987) who proposed that traditional SSD improvement programs assumed that 70% of plants will produce at least one seed. This efficiency is due to the abiotic and biotic stresses that are present during the crop period. Meanwhile the in vivo method is a controlled environment system so the efficiency was higher (76%) which allows the breeder greater flexibility in the generation of new improved materials. Similar results obtained O'Connor et al. (2013) who developed a speed breeding system in peanuts with efficiency between 68 and 74% for different segregating populations. Saxena et al. (2019) suggested that speed breeding could lead to the erosion of useful genetic variability. However, our data demonstrated that the speed breeding efficiency (in vivo methodology) was higher than the traditional SSD in the field.

It should be considerate that efficiency of 76% is produced by segregating population that have a high percentage of normal leaf plants that are less efficient than semi leafless (Tables 5 and 6). The architecture of the normal leaf plants, added to the high density (266 plants per m^2) used in this system, caused plant losses due to shading. Even though the efficiency achieved in vivo in segregating generations was adequate, could be increased crossing semi-leafless parental producing segregating populations 100% semi-leafless. These materials showed efficiency above 90% (Tables 5 and 6).

Semi-leaf cultivars are used by breeders and are demanded by producers for their benefits, in relation to the lower incidence of diseases and the lowest losses in

harvest, which makes it a very good opportunity to incorporate this methodology. However, in plant breeding often the normal leaf plants can be used as morphological markers to determine the success of the hybridization.

Speed breeding emerges as a technology that can be integrated with multiple disciplines (Chiurugwi et al. 2018). The combination of Speed breeding and Single Seed Decent has the potential to reduce the time required to develop new cultivars and increase the efficiency of breeding programs compared to conventional field systems. Another advantage is that it could be initiated in any moment of the year using a large amount of material in a small area (Cobb et al. 2019).

Conclusion

The identification of a system to accelerate generations by shortening each cycle is crucial in breeding programs. In the present work, in addition to comparing different methodologies, we present a simple, efficient and economic in vivo system that allows obtaining between 4.5 and 5.2 generations per year of peas.

Funding This research was funded by the National Council of Scientific and Technical Research (CONICET. Argentina), Agricultural Research Institute of Rosario (IICAR-CONICET) and Fund for Scientific and Technological Research (FONCyT. Argentina).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights No human or animal material was used. The research conducted complied with all institutional and national guidelines.

References

Atlin GN, Cairns JE, Das B (2017) Rapid breeding and varietal replacement are critical to adaptation of cropping systems in the developing world to climate change. Global Food Secur 12:31–37

Balzarini M, Gonzalez L, Tablada M, Casanoves F, Di Rienzo J, Robledo CW (2008) Infostat. Manual del Usuario. Editorial Brujas, Córdoba, Argentina

Barroso P, Crispim J, Costa M, Rêgo E (2019) How to shorten a plant-breeding program? A case study with ornamental pepper. Crop Breed Appl Biotechnol 19:193–199. https://doi.org/10.1590/1984-70332019v19n2a27

Bermejo C, Gatti I, Cointry E (2016) In vitro embryo culture to shorten the breeding cycle in lentil (Lens culinaris Medik). Plant Cell Tiss Organ Cult 127:585–590

Brummer FC, Barber WT, Collier SM, Cox TS, Johnson R, Murray SC, Olsen RC (2011) Plant breeding for harmony between agriculture and the environment. Front Ecol Environ Pratt A M Thro 9:561–568

Chahal GS, Gosal SS (2002) Principles and procedures of plant breeding: biotechnology and conventional approaches. Alpha Sci Int, London

Chiurugwi T, Kemp S, Powell W et al (2018) Speed breeding orphan crops. Theor Appl Genet. https://doi.org/10.1007/s00122-018-3202-7

Cobb JN, Juma RU, Biswas PS et al (2019) Enhancing the rate of genetic gain in public-sector plant breeding programs: lessons from the breeder's equation. Theor Appl Genet 132:627–645. https://doi.org/10.1007/s00122-019-03317-0

Collard BCY, Beredo JC, Lenaerts B et al (2017) Revisiting rice breeding methods—evaluating the use of rapid generation advance (RGA) for routine rice breeding. Plant Prod Sci 20:337–352. https://doi.org/10.1080/1343943X.2017.1391705

Croser JS, Ulsdorf M, Davies PA et al (2006) Toward doubled haploid production in the Fabaceae: progress, constraints, and opportunities. Crit Rev Plant Sci 25:139–157

Dagustu N, Sincik M, Bayram G, Bayraktaroglu M (2010) Regeneration of fertile plants from sunflower (*Helianthus annuus* L.) immature embryo. Helia 33(52):95–102

De La Fuente GN, Frei UK, Lübberstedt T (2013) Accelerating plant breeding. Trends Plant Sci 18:667–672. https://doi.org/10.1016/j.tplants.2013.09.001

Duc G, Bao SY, Baum M, Redden B, Sadiki M, Suso MJ, Vishniakova M, Zong XX (2010) Diversity maintenance and use of *Vicia faba* L. genetic resources. Field Crops Res 115:270–278

Eriksson D, Kershen D, Nepomuceno A, Pogson BJ, Prieto H, Purnhagen K, Smyth S, Wesseler J, Whelan A (2019) A comparison of the EU regulatory approach to directed mutagenesis with that of other jurisdictions, consequences for international trade and potential steps forward. New Phytol. https://doi.org/10.1111/nph.15627

FAOSTAT (2017) http://www.fao.org/faostat/es/#data/QC

Franklin G, Pius PK, Ignacimuthu S (2000) Factors affecting in vitro flowering and fruiting of green pea (*Pisum sativum* L.). Euphytica 115:65–74

Gamborg OL, Miller LA, Ojima K (1968) Nutrient requirements of suspension cultures of soyabean root cells. Exp Cell Res 50:151–158

Gebologlu N, Bozmaz S, Aydin M, Çakmak P (2011) The role of growth regulators, embryo age and genotypes on immature embryo germination and rapid generation advancement in tomato (*Lycopersicon esculentum* Mill.). Afr J Biotechnol 10:4895–4900

Germáná MA (2011) Anther culture for haploid and doubled haploid production. Plant Cell Tiss Organ Cult 104:283–300. https://doi.org/10.1007/s11240-010-9852-z

Ghosh S, Watson A, Gonzalez-Navarro OE et al (2018) Speed breeding in growth chambers and glasshouses for crop

breeding and model plant research. Nat Protoc 13:2944–2963
Goulden CH (1941). In: Proceedings of the 7th international congress genetics, vol 1039, pp 132–133
Greenway MB, Phillips IC, Lloyd MN et al (2012) A nutrient medium for diverse applications and tissue growth of plant species in vitro. Vitro Cell Dev Biol Plant 48:403–410. https://doi.org/10.1007/s11627-012-9452-1
Hickey LT, Hafeez NA, Robinson H et al (2019) Breeding crops to feed 10 billion. Nat Biotechnol 37(7):744–754. https://doi.org/10.1038/s41587-019-0152-9
Idrissi O (2020) Application of extended photoperiod in lentil: towards accelerated genetic gain in breeding for rapid improved variety development. Mor J Agric Sci 1(1):14–19
Idrissi O, Sahri A, Udupa S, Kumar Sh (2019) Single seed descent under extended photoperiod as a simple, rapid and efficient breeding method for accelerated genetic gain in lentil. In: Proceedings of the 3rd international legume society conference, Poznan, Poland, 21 24/05/2019
Jensen ES, Peoples MB, Boddey RM et al (2012) Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. Agron Sustain Dev 32:329–364. https://doi.org/10.1007/s13593-011-0056-7
Knauft DA, Norden AJ, Gorbet DW (1987) Peanut. In: Principles of cultivar development, vol 2. Crop Species. Macmillian Publishing, New York, pp 346–385
Liu H, Zwer P, Wang H, Liu C, Lu Z, Wang Y, Yan G (2016) A fast generation cycling system for oat and triticalebreeding. Plant Breed 135(5):574–579
Lulsdorf MM, 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 Breed 137:486–491. https://doi.org/10.1111/pbr.12612
Mobini SH, Warkentin TD (2016) A simple and eficient method of in vivo rapid generation technology in pea (*Pisum sativum* L.) in vitro. Cell Dev-Pl 52:530–536
Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assayswith tobacco tissue cultures. Physiol Plant 15:473–497
Nagatoshi Y, Fujita Y (2018) Accelerating soybean breeding in a CO_2-supplemented growth chamber. Plant Cell Physiol 60:77–84. https://doi.org/10.1093/pcp/pcy189
Nelson GC, Rosegrant M, Palazzo A et al (2010) Food security, farming, and climate change to 2050: scenarios, results, policy options. International Food Policy Research Institute (IFPRI), Washington, p 132
O'Connor DJ, Wright GC, Dieters MJ, George DL, Hunter MN, Tatnell JR, Fleischfresser DB (2013) Development and application of speed breeding technologies in a commercial peanut breeding program. Peanut Sci 40:107–111
Ochatt SJ, Sangwan RS (2010) In vitro flowering and seed set: acceleration of generation cycles. In: Davey MR, Anthony P (eds) Plant cell culture: essential methods. Wiley, Chichester, pp 97–110
Ochatt S, Sangwan RS, Marget P et al (2002) New approaches towards the shortening of generation cycles for faster breeding of protein legumes. Plant Breed 121:436–440
Rademacher W (2000) Growth retardants: effects on gibberellins biosynthesis and others metabolic pathways. Annu Rev Plant Physiol Plant Mol Biol 51:501–531. https://doi.org/10.1146/annurev.arplant.51.1.501
Ribalta FM, Croser JS, Erskine W, Finnegan PM, Lulsdorf MM, Ochatt SJ (2014) Antigibberellin-induced reduction of internode length favors in vitro flowering and seed-set in diferent pea genotypes. Biol Plant 58:39–46. https://doi.org/10.1007/s10535-013-0379-0
Ribalta FM, Pazos-Navarro M, Nelson K et al (2017) Precocious floral initiation and identification of exact timing of embryo physiological maturity facilitate germination of immature seeds to truncate the lifecycle of pea. Plant Growth Regul 81:345–353. https://doi.org/10.1007/s10725-016-0211-x
Samineni S, Sen M, Sajja SB, Gaur PM (2019) Rapid generation advance (RGA) in chickpea to produce up to seven generations per year and enable speed breeding. Crop J 8:164–169. https://doi.org/10.1016/j.cj.2019.08.003
Saxena KB, Saxena RK, Hickey LT, Varshney RK (2019) Can a speed breeding approach accelerate genetic gain in pigeonpea? Euphytica 215:202. https://doi.org/10.1007/s10681-019-2520-4
Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52:591–611. https://doi.org/10.1093/biomet/52.3-4.591
Sita K, Sehgal A, HanumanthaRao B et al (2017) Food legumes and rising temperatures: effects, adaptive functional mechanisms specific to reproductive growth stage and strategies to improve heat tolerance. Front Plant Sci 8:1658. https://doi.org/10.3389/fpls.2017.01658
Surma M, Adamski T, Święcicki S et al (2013) Preliminary results of in vitro culture of pea and lupin embryos for the reduction of generation cycles in single seed descent technique. Acta Soc Bot Pol 82(3):231–236. https://doi.org/10.5586/asbp.2013.021
Varshney RK, Pandey MK, Bohra A et al (2019) Toward the sequence-based breeding in legumes in the post-genome sequencing era. Theor Appl Genet 132:797–816. https://doi.org/10.1007/s00122-018-3252-x
Watson A, Ghosh S, Williams M et al (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants 4:23–29