Plant Methods

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Rapid Ragi: A speed breeding protocol for finger millet

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Abstract

Background Climate change is gradually increasing demand for resilient, nutritious crops like finger millet or ragi. Ensuring food security requires researchers to develop improved and adapted cultivars rapidly. Modern techniques such as genomics-assisted breeding have emerged in the previous decade and combined with rapid generation advancement they will offer a step change in the speed of cultivar development.

Results In this study, we developed a repeatable and cost-effective speed breeding protocol for finger millet by modulating the agronomic and physiological components for early generation advancement. A photoperiod of 9-hours, 29 ± 2℃ temperature, 70% relative humidity, 105 plants per 1.5 sq. ft., 0.17% Hoagland's No. 2 solution spray, restricted irrigation and harvesting at physiological maturity successfully reduced 28–54 days across the maturity groups of finger millet. The advantage was validated in segregating populations confirming up to 4–5 generations a year, instead of 1–2 under field conditions.

Conclusion The speed breeding protocol developed reduces the breeding cycle time significantly allowing increased genetic gain. The protocol provides the advantage of rapid development of recombinant inbred lines (RILs), high-throughput phenotyping for biotic and abiotic stresses, and genotyping for early generation selections.

Keywords Finger millet, Speed breeding, Photoperiod, Rapid generation advancement, Short-day plant, High-density planting

Background

Crop diversification and food security are two major challenges in the current era. Recent crop model reports project an earlier impact of climate change by 2040 with a substantial decrease (> 5%) of productivity in rice, wheat, maize, and soybean mainly in the arid zones of the world [1, 2]. The decrease in available arable land, while needing an increased food production to feed growing population, is creating demand for crops that flourish in areas where the big three staples are gradually unable to grow. These resilient crops should be able to produce nutrient-dense food from meagre resources at the same time. In this context, millets are gaining popularity for their climate-resilience and finger millet (*Eleusine coracana* L.; also known as 'ragi') is one such crop, which is well-adapted to marginal agricultural lands.

Millions of people rely on finger millet as a staple, and it is commonly cultivated in semi-arid parts of Eastern and Southern Africa and South Asia [3]. Uganda, Ethiopia, India, Nepal, and China are the world's top finger

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millet-producing countries. The yearly global output of finger millet is estimated at around 3.70 million tonnes from a total area of about 2.20 million hectares with a productivity of 1.32 tonnes per hectare [4]. It is the third most important millet crop after sorghum and pearl millet and the most important small millet in the tropics covering 12% of the global millet area. In India, finger millet ranks first in importance amongst small millets with a share of 81% of the small millets produced [5]. In India, it covers one million hectares, with a total yield of 1.76 million tonnes and average productivity of 1.74 tonnes per hectare [4].

Finger millet is an allotetraploid C_4 plant capable of thriving in fluctuating environmental conditions due to its ability to grow in various habitats, from coastal plains to highlands and at elevations of 500–2400 meters above mean sea level [6, 7]. It is highly regarded for its nutritional value, especially calcium, dietary fibre, and phenolic compounds among others. Additionally, it is an important source of iron, methionine, and other amino acids, as well as slowly digestible starch and polyphenols [8, 9]. Its gluten-free, low-fat, and easily digestible nature makes it suitable for those with dietary restrictions or sensitivities, earning it the title of "super cereal" [3].

While it has several benefits, research efforts are minimal compared to rice, wheat, and maize. Rising health concerns and climate change challenge breeders to achieve crop improvement faster. Conventionally, the development of improved lines in finger millet involves 4–5 years of breeding and 4–5 years of testing including 1–2 years of multi-environment trials (for promising breeding lines) and three years of national testing (for nominated entries). Advanced tools such as molecular markers, mapping populations, and phenomics [10] can provide impetus for early line development, however, higher genetic gains require a substantial reduction of breeding cycle time, alongside accurate selection for desirable genotypes. Rapid generation advancement, also known as 'speed breeding', is one of the methods to reduce the breeding cycle time.

Speed breeding forms the simplest solution to reducing the generation time in a crop. Speed breeding involves tuning of photoperiod, light intensity, light quality, temperature, and relative humidity to physiologically promote early flowering and maturity in plants [11]. It has been successful in various crops including, rice, wheat, barley soybean, brassica, chickpea and pigeonpea for rapid generation advancement [12–18]. Combining the speed breeding approach with agronomic interventions allowed us to create a robust, efficient, and economical platform for finger millet breeding. This study focuses on optimizing the protocol for finger millet rapid generation advancement while keeping it cost-efficient and practical.

Materials and methods

Plant material

Accessions comprising released varieties and germplasm lines obtained from the Gene bank, ICRISAT were utilized for the experiments. A set of 9 accessions were utilised for standardising the plant density, 5 for standardising the nutrient and photoperiod, 4 for optimising the protocol and 10 for validation of the protocol. Additionally, the protocol was validated in 3 segregating populations. The details of the accessions and their phenological traits are presented in Supplementary Table 1.

Speed breeding facility

The experiments were conducted in an established rapid generation advancement facility at ICRISAT measuring 208 sq. m. (26 m × 8 m) including an anteroom of 6 m x 8 m that houses the control panel and dosatron system for supplying nutrients. The facility consists of 10 workbenches with 30 plastic trays (Make: Sri Sai Fibres Pvt Ltd.) of size 2.5 m × 1.21 m. These trays were fitted with ebb-and-flow (flood and drain) irrigation facility. Each tray can accommodate either 18 '105-wells' or 16 '50-wells' nursery trays. Each well houses one plant.

Nursery trays for high density planting

Nursery trays of 50-wells and 105-wells were used for high density planting in finger millet. The trays were made of high impact polystyrene (HIPS) material (locally manufactured) measuring 53 cm in length and 27 cm in width with a depth of 3 cm. Bottom-up irrigation was supported through a hole at the bottom of each well. These trays were filled with a mixture of soil, sand, and vermicompost in a 3:2:1 ratio. One seed was placed in each well and covered with a thin layer of fine sand for easy germination.

Light requirements

The artificial light requirements were met with non-tuneable visible spectrum LED 9-watt bulbs (Make: LUKER). Two bulbs per tray were arranged at an adjustable height of 40–45 cm above the trays. The germination and vegetative stages were exposed to ambient light at day lengths, while the artificial light treatment was initiated post 4–5 leaf stage or when the plant attained a height of 25–30 cm. The photoperiods tested were 5-hours, 7-hours, 8-hours, 9-hours, and 13-hours in different experiments. The photoperiod was initiated at 8am and ended at 1pm (5-hours), 3pm (7-hours), 4pm (8-hours), 5pm (9-hours), and 9pm (13-hours). The short-day photoperiod was provided by covering the plants with a black polythene sheet of 1 mm thickness on all sides. A 1-inch gap from the rim of tray on one side (perpendicular to the light source) was left for aeration. The photoperiod

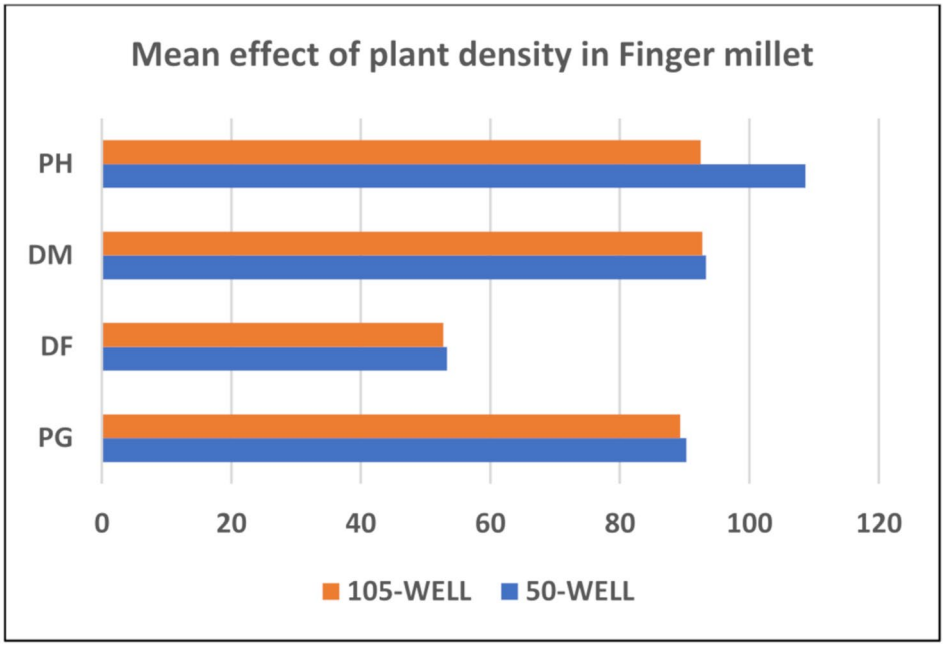

Fig. 1 Mean effect of planting density on finger millet
* PH: *Plant height* (cm); DM: *Days to maturity*; DF: *Days to 50% flowering;* PG: *Percentage of Germination* (%)

requirements were met with the ambient light from 8am to 3pm followed by the external light source (bulbs).

Temperature and humidity

Temperature was set at 29 ± 2℃ with a relative humidity of 70%. This was maintained using a programmable logic controller (PLC, Make: SCHNEIDER) with a range of 20℃ to 60℃. Cooling was provided through a honeycomb cooling pad system (Make: Sri Sai Fibres Pvt Ltd.), while heating was provided through heaters.

Irrigation and nutrition

Irrigation was provided through the ebb-and-flow system for 5–6 min every alternate day. Hoagland's No.2 solution (a formulated combination of macronutrients and micronutrients, (Make: HIMEDIA) at 0.17%, Basacote (Make: DHANASHREE AGRO INDUSTRIES) + NPK (20:20:20, Make: NUTRIFEED) at 1% were tested as spray formulations. The NPK (19:19:19, Make: NUTRIFEED) at 0.2%, supplementation was done when the leaves exhibited pale green colour. The application resulted in healthy green leaves. The spray technique was preferred over the fertigation to avoid nutrient losses (Supplementary Table 2).

Results

Standardizing the components of protocol

Planting density

Eight accessions of finger millet were grown in 50-well (50 plants in 1.5 sq.m) and 105-well (105 plants in 1.5 sq.m) trays in two replications keeping all other factors constant as a pilot experiment to assess the growth and vigour of the plants. The genotypes were compared for percentage of germination (PG), days to 50% flowering (DF), days to maturity (DM), and plant height in cm (PH) (Supplementary Tables 3 and 4). The results showed a minimum germination of 83% except for IE 501 in both the planting densities. The differences in phenological traits (*days to 50% flowering* and *days to maturity*) were non-significant among the planting densities tested, while plant height was significantly lower when planted in 105-well trays. These results led us to adopt 105-well tray for planting purposes, which also proved to be cost-effective (Fig. 1; Table 1).

Table 1 Earliness of finger millet genotypes through high-density planting

	50-well tray		105-well tray	
	DF	DM	DF	DM
Early	16	7	16	7
Medium	25	31	26	32
Long	28	31	28	31
Average	23	23	23	23

* DF: *Days to 50% flowering*; DM: *Days to maturity*

Nutrition and photoperiod requirements

The next experiment focussed on standardizing the nutrition requirements as an essential agronomic intervention. This was combined with photoperiod treatments to induce early flowering. Two nutrient treatments comprising Hoagland's No.2 solution at 0.17% and

Basacote + 20:20:20 at 1% formulations were tested at 5-hours, 7-hours, and 9-hours photoperiods on five finger millet genotypes. The results showed a significant difference in *days to 50% flowering* across the photoperiods and nutrient formulations. A photoperiod of 9-hours stimulated early flowering followed by 7-hours and 5-hours. The genotypes with Hoagland's No.2 solution treatment responded positively (earliness) for phenological traits compared to Basacote + 20:20:20 (Fig. 2, Supplementary Tables 5 and 6). KMR 301 for an unknown reason did not flower across the photoperiods under Basacote treatment, while in contrast showed early flowering with Hoagland's solution.

Testing the protocol

The protocol was tested for all the parameters during post-rainy (Rabi) season 2023, while extending the experiment up till harvest maturity. The factors (planting density, nutrients, and photoperiod) were tested on finger millet genotypes covering early (V1), medium duration (V2, V3), and long duration (V4) segments. The mean performance and analysis of variance for the tested parameters and their interactions are presented in Supplementary Tables 7 and 8. The analysis of variance showed significant variance for *days to 50% flowering*, *days to maturity*, *plant height* (cm), and *seeds per panicle* upon the interaction of nutrient × photoperiod × genotypes. A 9-hours photoperiod with a Hoagland's No.2 solution spray at 0.17% and 105-well plant density (1.5 sq. ft.) resulted in the earliest response with mean *days to 50% flowering* of 52.6 days and mean *days to maturity* of 68.6 days. This resulted in a reduction of 31 and 51 days, respectively, in flowering and maturity over the control with a maximum of 48 and 71 days, respectively, in Variety 4. The performance of genotypes at 8-hours and 9-hours was found to be similar for earliness. Comparatively, the 9-hours combination showed a desired lower plant height and a higher *seed number per panicle* (Fig. 3; Table 2). The harvested seeds exhibited a 100% germination rate.

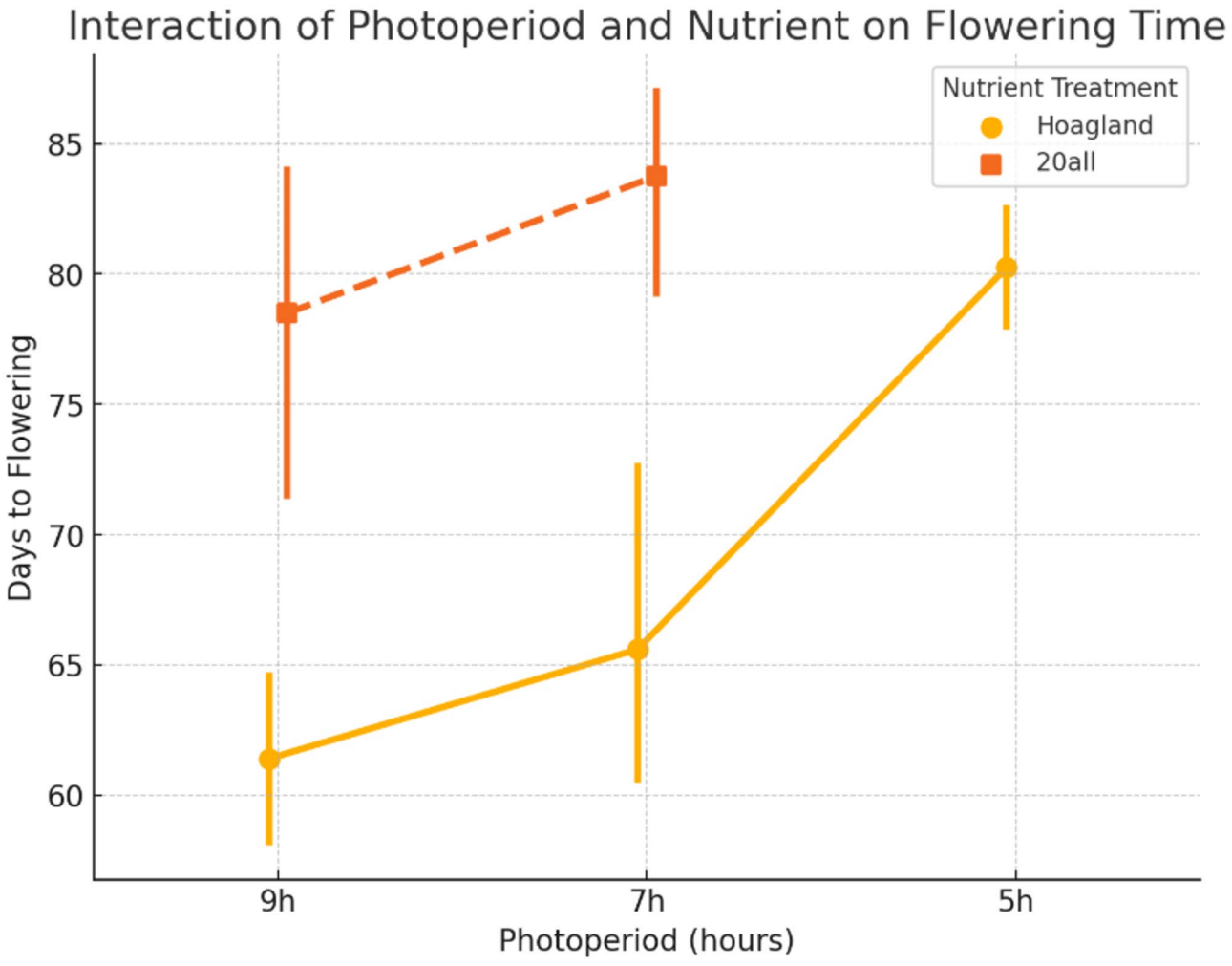

Fig. 2 Effect of nutrient and photoperiod treatments on flowering time of Finger millet genotypes

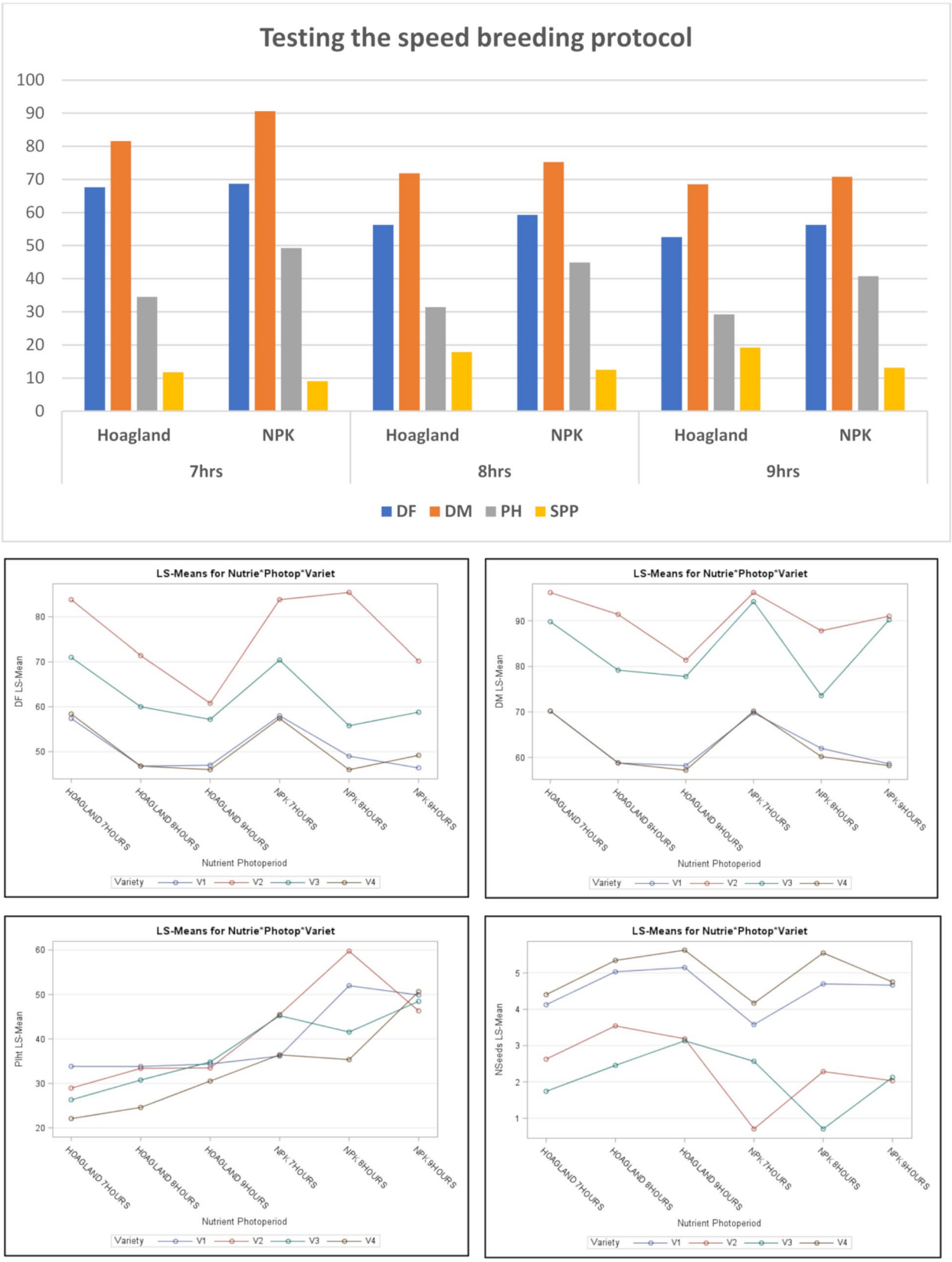

Fig. 3 Testing the speed breeding protocol on finger millet genotypes depicting the mean effect of photoperiod and nutrient combinations
*DF: *Days to 50% flowering*; DM: *Days to maturity*; PH: *Plant height (cm)*; SPP: *Seeds per panicle*

Table 2 Reduction in days obtained in the finger millet genotypes compared to the control after the treatments

Parameter	DF	DM
Hoagland*7hrs*V1	12.30	37.80
Hoagland*7hrs*V2	2.20	19.80
Hoagland*7hrs*V3	15.30	35.20
Hoagland*7hrs*V4	36.10	57.80
Mean	**16.48**	**37.65**
Hoagland*8hrs*V1	22.10	49.20
Hoagland*8hrs*V2	7.60	24.60
Hoagland*8hrs*V3	28.10	45.80
Hoagland*8hrs*V4	47.60	69.20
Mean	**26.35**	**47.20**
Hoagland*9hrs*V1	23.00	49.80
Hoagland*9hrs*V2	25.20	34.60
Hoagland*9hrs*V3	28.80	47.20
Hoagland*9hrs*V4	48.00	70.80
Mean	**31.25**	**50.60**
NPK*7hrs*V1	12.00	38.20
NPK*7hrs*V2	2.20	19.80
NPK*7hrs*V3	15.60	30.80
NPK*7hrs*V4	36.60	57.80
Mean	**16.60**	**36.65**
NPK*8hrs*V1	21.00	46.00
NPK*8hrs*V2	0.60	28.20
NPK*8hrs*V3	30.20	51.40
NPK*8hrs*V4	48.00	67.80
Mean	**24.95**	**48.35**
NPK*9hrs*V1	23.60	49.40
NPK*9hrs*V2	15.80	25.00
NPK*9hrs*V3	27.20	34.80
NPK*9hrs*V4	44.80	69.80
Mean	**27.85**	**44.75**

*DF: *Days to 50% flowering*; DM: *Days to maturity*

Validation of the protocol with an added early harvest

The protocol was validated on a set of 10 released varieties spread across the maturity groups (early, medium and long). The set was validated in rainy (*kharif*) season 2024 with Hoagland's No. 2 solution spray at 0.17% and 105-well plant density at 7-, 8-, 9-, and 13-hours photoperiods. The results showed a major advantage of up to 26, 35, 35, and 13 days at 7-, 8-, 9-, and 13-hours photoperiod, respectively. An 8- or 9-hours photoperiod resulted in maximum benefit with 21, 37–38, and 45-46-days reduction in maturity in early, medium and long duration genotypes, respectively (Table 3).

The stages of the genotypes were also tracked to identify the right stage of harvest at dough stage, physiological maturity, and harvest maturity. The results showed 95–100% germination at physiological maturity, allowing an additional 6–8-day reduction in maturity date, through early harvest. The photoperiods of 8 or 9-hours with harvesting at physiological maturity caused genotypes with average harvesting dates of 100–135 days to be harvested at 75–78 DAS. The mean values of the individual genotypes across the stages are presented in (Fig. 4, Supplementary Table 9).

Rapid-Ragi makes 4–5 generations possible in a year

The developed protocol was also tested for advancing segregating populations in the rainy (*Kharif*) season 2024. Random breeding lines from cross combinations consisting of parents from medium and long-duration segments were compared using the developed protocol. The results were noteworthy with the F_2 and F_3 populations flowering and maturing within 42–48 and 72–75 days after sowing per generation, respectively. Additionally, early harvest at physiological maturity and single seed descent saves 7–8 days, thus completing the life cycle within 64–68 days after sowing. The breeding lines displayed a 100% germination rate, showing the intact vigour of the plants with an out-turn of 80–150 seeds from 2 to 4 fingers per panicle. This implies the ability of the protocol to complete 4–5 generations in a year in comparison to 1–2 generations under field conditions (Table 4).

Table 3 Mean performance of finger millet genotypes with speed breeding protocol

Maturity class	Control DM	Days to maturity in Speed breeding facility							
		7-hours		8-hours		9-hours		13-hours	
		PM	HM	PM	HM	PM	HM	PM	HM
Early	104	84.85	91.85	77.2	82.2	75.35	82.5	97.5	105.35
Medium	123	87.46	94.46	78.26	84.38	77.52	85.44	99.46	107.4
Late	132	87.97	94.97	80.00	86.77	78.23	86.10	99.9	107.9
Maturity	**Control DM**	**Number of days reduced**							
		7-hours		8-hours		9-hours		13-hours	
		PM	HM	PM	HM	PM	HM	PM	HM
Early	104	18.65	11.65	26.3	21.3	28.15	21	6	-1.85
Medium	123	35.34	28.34	44.54	38.42	45.28	37.36	23.34	15.4
Late	132	44.03	37.03	52.00	45.23	53.77	45.90	32.10	24.10
AVERAGE	119.67	32.67	25.67	40.95	34.98	42.40	34.75	20.48	12.55

*PM- *Physiological Maturity*; HM- *Harvest Maturity*

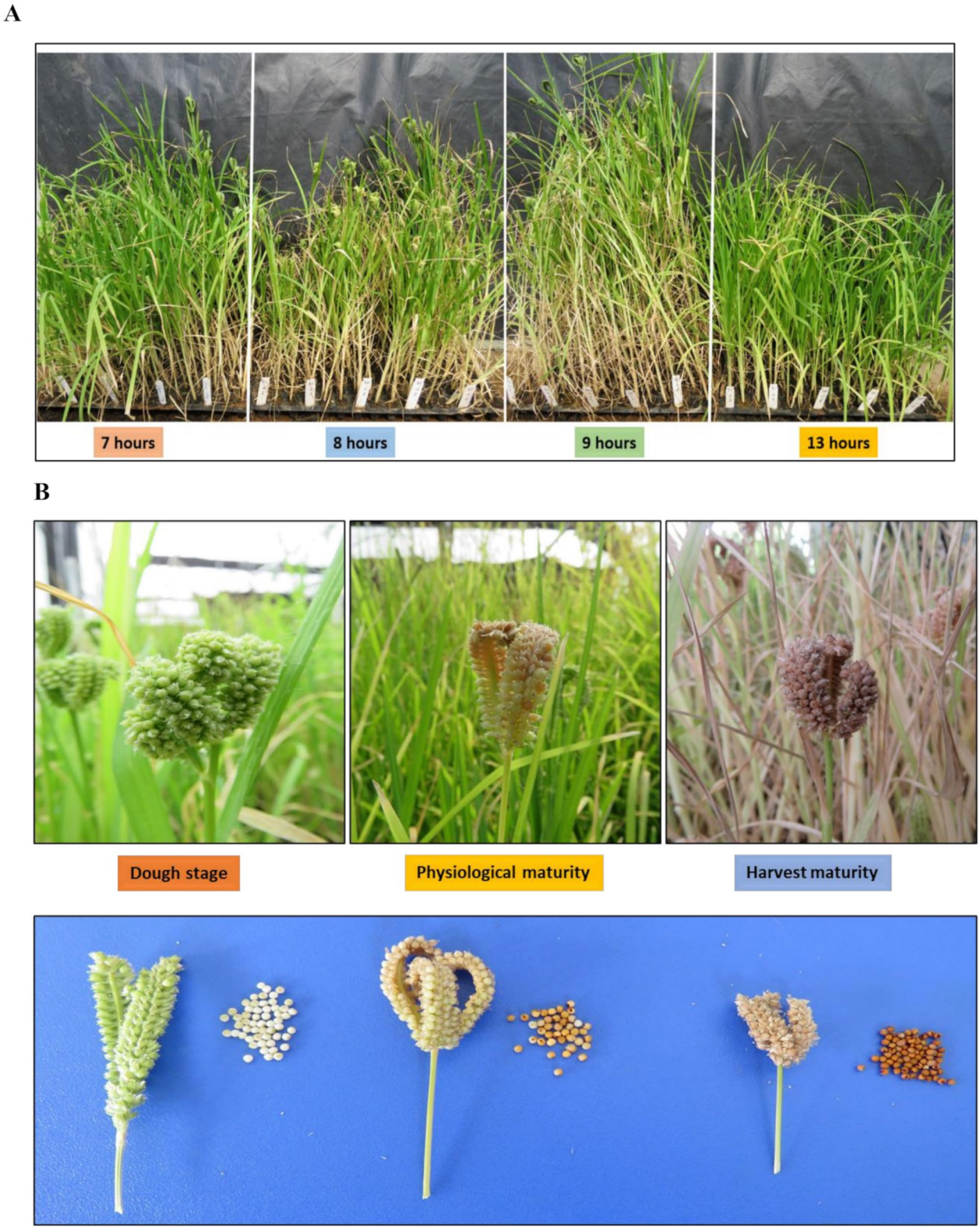

Fig. 4 Finger millet genotype performance at various photoperiods under regulated plant density and nutrition**(A)**. Stages of finger millet seed formation **(B)**

Table 4 Mean performance of finger millet segregating generations with rapid generation advancement protocol

Cross combination	DF- Female parent*	DF- Male parent*	F2 population		F3 population	
			DF	DM	DF	DM
ICFX221056	89	77	41–45	66–71	46–47	73–74
ICFX221039	82	72	41–43	66–68	43–44	67–68
ICFX221062	83	60	45–47	70–71	50–52	74–75

Note: * indicates the data collected under conventional field conditions

Discussion

The "International Year of Millets, 2023" created the demand for nutritious millets to be brought back into the food chain, while it also posed a challenge to breeders to deliver improved cultivars more quickly. Finger millet matures in approximately 100–135 days, with 5–6 days for germination, 55–70 days for vegetative growth, 20–25 days for flowering, and another 25–40 days for maturation. This timeline provides the possibility of 1–2 seasons a year. Adopting rapid generation advancement (RGA) technology in finger millet requires the convergence of agronomy, physiology and controlled environmental conditions to shorten every stage of plant growth.

High density planting (HDP) of finger millet in the speed breeding facility serves a dual advantage of optimising space, resources and reducing costs. A speed breeding facility of 208 m^2 (26 m × 8 m) can accommodate around 56,700 plants at the optimised high density, while the same requires 1701 m^2 under field conditions (at a standard spacing of 30 × 10 cm). Due to high density planting, the plants will be delicate and lanky for need of light. If they grow tall (beyond 50–55 cm), they tend to lodge. The 105-well tray provided the required height and healthy plant growth. Furthermore, soil, water, and nutrient supply was designed to stress plants, without affecting seed viability at harvest.

Literature studies mention the short-day nature of finger millet [19] yet no reports on the exact photoperiod requirements of the crop have been published. The initial experiments for standardizing nutrient and photoperiod confirmed the short-day nature. Additionally, it provided insights into the varying degrees of photoperiod sensitivity based on the maturity of the tested genotypes at different light periods. Speed breeding protocols were successfully formulated in short-day plants including rice, soybean and pigeonpea where light spectrum and intensity are the deciding factors. Those efforts were majorly directed towards shortening the vegetative phase of the crop [12, 13, 20, 21]. In contrast, finger millet does not require specific light wavelengths across the different growth stages and adds the advantage of significant earliness in vegetative, flowering and maturation phases. The protocol was successful with ambient light conditions supplemented with additional light for 1–2 h and on cloudy days. This forms the core of making this protocol economical and easily repeatable. Temperature is another important factor affecting the growth rate of finger millet. Based on the optimum temperatures of 28–32℃ suggested for finger millet, temperature was maintained at 29 ± 2℃ with a controlled system of cooling pads and heaters [22].

A 9-hours photoperiod was found to be the best treatment compared to 7- and 5-hours of light. The results at 5-hours light period gave no advantage over the control. This suggests the possibility of insufficient light. Hence, imposing a minimum of 7-hours light is likely to be necessary in finger millet. Additionally, 5-hours photoperiod showed no sign of flowering with Basacote + 20:20:20 which suggests a contribution of Hoagland's No.2 solution in flowering. The similar advantage of using Hoagland's solution was noted in genotype KMR 301 under 7- and 9-hours photoperiods. Hoagland's No. 2 solution provides a well-balanced mix of essential macro- and micronutrients, ensuring optimal plant growth and faster developmental transitions. The hydroponic nature of Hoagland's solution allows precise nutrient uptake, preventing deficiencies that could delay flowering.

The protocol was further optimised and validated in a range of germplasm and released cultivars, respectively, by narrowing down using the photoperiods 7-, 8-, and 9-hours. The 8- and 9-hours photoperiods showed similar earliness, yet a 9-hours photoperiod is recommended taking into consideration the seasonal variances as the ambient light intensities vary across the seasons. These experiments were conducted in post-rainy and rainy seasons, respectively (Latitude: 17.512; Longitude: 78.275) across the maturity duration range as they are the preferred seasons for finger millet cultivation. Both the experiments recorded the potential of short duration photoperiods combined with the agronomic interventions. Studies report the benefit of pre-mature harvest for hastening the breeding cycle [3, 13]. This was attempted in finger millet genotypes harvesting and germinating the seeds at dough stage (50–60%), physiological (95–100%) and harvest maturity (100%). Based on the results, a photoperiod of 9-hours with 105-well plant density, Hoagland's No. 2 solution spray at 0.17% at a temperature of 29 ± 2℃, relative humidity of 70%, with harvest at physiological maturity forms the *Rapid Ragi* protocol for rapid generation advancement in finger millet (Table 5). On average, across the seasons, an advantage of 29–57 days in early, 47–48 days in medium, and 56–78 days in

Table 5 *Rapid Ragi* protocol for finger millet

Parameter	Details
Planting density	105-wells tray placed on mat
Depth of well	1 inch
Soil composition	3:2:1 of soil: sand: vermicompost
Nutrients	Spray of Hoagland's No. 2 solution at 0.17% at an interval of 3 days up to vegetative stage followed by a 5-day interval
Irrigation	EBB-and-flow method on every alternate day for 5–6 min
Light source	LED 9-watt bulb
Light duration	9-hours photoperiod
Light treatment	Maintained by covering the plants with a black polythene sheet of 1 mm thickness with a 1-inch gap from the base for aeration on one side of the trays (perpendicular to light source)
Distance from light source	40–45 cm (3 bulbs for a tub size of 1.25 m × 1.5 m or 18 trays)
Time of treatment	Plant height of 25–30 cm or 4–5 leaf stage
Temperature	29 ± 2℃
Relative Humidity	70%
Output	80–150 seeds from 2–4 fingers per panicle with a 95–100% germination

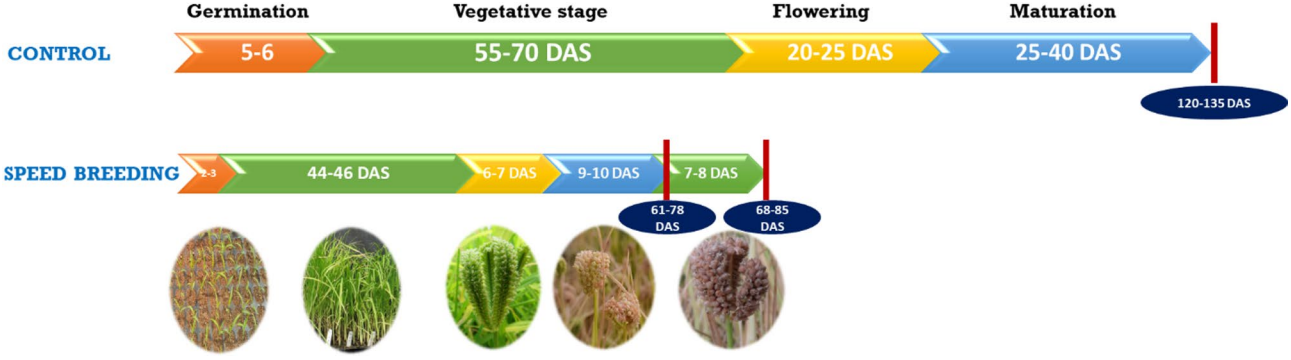

Fig. 5 Advantage of speed breeding protocol over field conditions

long duration genotypes was possible. Thus, across the durations and seasons finger millet genotypes with early harvest can be advanced within 61–78 days at maturity (Fig. 5).

The current finger millet breeding pipeline is bound by the seasonal barrier with a maximum of 1–2 seasons/year, with slow crop establishment, high weed competition, laborious field management for generation advancement and high associated costs. *Rapid-Ragi* protocol is designed to attain early harvests at low-cost of resources with easy repeatability. The reduction in cost is obtained with the lower seed rate, higher plant density, 1/8th space requirements, dependence on ambient light, low-cost light sources and no weed management required compared to field conditions. The population advancement study done confirmed 4–5 generations per year with a 100% intact progeny emergence thus enhancing the potential rate of genetic gain. This protocol could allow easily integrated with marker assisted selection, gene pyramiding, rapid trait introgression and genomic selection to enhance precision in breeding programs.

Conclusion

The present study is aimed at developing a speed breeding protocol for finger millet using variable photoperiods, temperature, and relative humidity along with agronomical interventions such as high-density planting and controlled water and nutrient supply. A photoperiod of 9-hours, 29 ± 2℃, 70% relative humidity, 105 plants per 1.5 sq ft., 0.17% Hoagland's No. 2 solution spray, restricted irrigation and early harvest at physiological maturity reduced 29–57 days in early, 47–48 days in medium, and 56–78 days in long duration varieties of finger millet. The *Rapid Ragi* protocol was applied to segregating populations in two successive generations which confirmed the results. With this new speed breeding protocol, it is possible to have 4–5 generations in a year.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13007-025-01403-7.

Supplementary Material 1

Acknowledgements
The authors would like to express their sincere gratitude to the leadership of ICRISAT for giving us the opportunity to conduct this study. The help and timely support from the Farm Engineering Services is sincerely appreciated.

Author contributions
SS, JP, PR, SP: Planning of speed breeding protocol. JP, S, KSV, RR, YJ, PJ: Execution and optimization of speed breeding protocol. SP, CVS: Nutrient advisory. JP, PR, RR: Data analysis, Prepared tables and figures. SS, JP: Manuscript development. SS, CVS, SM: Reviewing of Manuscript.

Funding
No funding support.

Data availability
Data is provided within the manuscript and supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Authors declare consent for publication.

Competing interests
The authors declare no competing interest.

Received: 8 April 2025 / Accepted: 5 June 2025
Published online: 12 June 2025

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