Indian J. Genet. Plant Breed., (2025); 85(1): 35-42
https://doi.org/10.31742/ISGPB.85.1.4
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Rapid generation advancement protocol of wheat under natural day-length conditions during summer seasons in sub-tropical conditions

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Abstract

Developing improved crop cultivars is essential for confronting adverse environmental conditions. Rapid cultivar development requires reducing crop duration through rapid generation advancement (RGA) strategies. Extreme conditions that allow five to six crops annually may not provide plant breeders the opportunity for selection based on phenological traits and also necessitate a well-equipped growth chamber. The present study aimed to develop an RGA protocol under natural daylight during the summer season in sub-tropical conditions, incorporating irrigation, and plant density modulation alongside the chilling treatment of harvested seed. Wheat cultivars HD2932 and HD3086 were grown in 4-inch plastic pots with either one or four seeds per pot. The plants grown at higher density flowered 3 to 4 days earlier than their low-density counterparts. Irrigation was stopped either at 15 or 25 days after anthesis for forced maturity in different sets of plants, and the matured seeds were harvested after 20 days of the last irrigation. Consequently, the seed-to-seed cycle was shortened to 90 to 95 days compared to the regular cropping season (140–150 days). The seeds were subjected to chilling treatment at 4°C prior to sowing, and it was observed that chilling treatment for up to 72 hours can increase germination percentage beyond 90%. The protocol standardized in the current study can help to get two wheat crops with only temperature-controlled chambers in sub-tropical conditions during the summer season. The method can be used intermittently by growing two summer crops and a rabi crop, permitting selection under intermittent moderate RGA and natural growing conditions.

Keywords: Rapid generation advancement, wheat, natural day-length, limited moisture, chilling treatment

Introduction

The global research community is confronted with significant challenges pertaining to the accelerated growth of the human population, which is anticipated to attain 9.7 billion by the year 2050 and 10.4 billion by the year 2100 (UN DESA 2019). As crop production and the availability of food grains face pressure to meet the rising demands, this presents a challenging task for plant breeders to develop high-yield varieties. Currently, crop yields increase by approximately 1.3% per year, which is only about half of the expected requirement. The land under cultivation is not anticipated to increase, which is concerning, given the uncertainties about declining agricultural lands and shifting climatic conditions (Hemathilake and Gunathilake 2022). Therefore, it is a massive task for breeders and scientists to feed an ever-growing population with limited agricultural lands and erratic climatic conditions. To address future food and nutritional security concerns, it is essential to develop and implement sustainable breeding tools that enable us to achieve a higher yield of crops per unit of

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How to cite this article: Raj N., Shukla H., Bhurta R., Bijarania S., Kapoor U., Chandra A.K., Agarwal P., Raghunandan K., Mallick N., Niranjana M., Kumar M.B.A., Sathee L. and Jha S.K. 2025. Rapid generation advancement protocol of Wheat under natural day-length conditions during summer seasons in sub-tropical conditions. Indian J. Genet. Plant Breed., **85**(1): 35-42.

Source of support: ICAR-IARI, Govt. of India, New Delhi

Conflict of interest: None.

Received: May 2024 **Revised:** Dec. 2024 **Accepted:** Jan. 2025

land (Chandra et al. 2022). Introducing crop varieties suited to new environmental conditions or cultivating different crop species can help mitigate some of the impacts of climate change. Both of these solutions require rapid breeding processes. The time it takes to develop a crop variety primarily depends on the years required to develop homozygous lines after hybridization. Typically, it takes 7 to 9 years to achieve homozygosity and develop a cultivar if only one crop generation is produced annually. To expedite this process, doubled haploids (DH) or rapid generation advance (RGA) techniques are employed to reduce the number of years required to develop a cultivar (Watson et al. 2018).

Bread wheat (*Triticum aestivum* L.) is a vital cereal that sustains the global population, contributing one-fifth of the world's dietary calorie and protein intake (Mallick et al. 2022; Bhurta et al. 2023). As the second most widely grown crop, wheat is expected to play a crucial role in feeding an ever-growing population (Raghunandan et al. 2022). Wheat production must increase from 1% to 1.7% annually to achieve the target of 1 billion tonnes production by 2050 (Tadesse et al. 2019). Breeding new, advanced, and improved wheat cultivars can often take several years when using conventional breeding methods. Selecting a donor parent and initiating the crossing process typically requires 4 to 6 generations of inbreeding to develop genetically stable homozygous lines. This can be time-consuming and labor-intensive for most field crops, as they generally allow for only 1 to 2 generations per year. The recently developed SMART breeding technology, commonly referred to as "speed breeding," offers rapid generation advancement of segregating lines to achieve the necessary homozygosity. RGA technology has proven effective in accelerating breeding cycles and advancing breeding progress in numerous crops (Watson et al. 2018; Pandey et al. 2022; Adhikari et al. 2023).

Speed breeding techniques manipulate the environmental conditions under which crop genotypes are grown to achieve accelerated flowering and seed sets as quickly as possible. Changes in environmental conditions strongly influence plant growth, development, and reproductive behavior. The impact of environmental factors such as temperature, photoperiods, humidity, and others on seed germination, shoot elongation, lateral branching, flowering, and seed set in cereal crops has been extensively studied (Watson et al. 2018; Samineni et al. 2020; Kigoni et al. 2023). By employing effective selection and RGA protocols, reducing breeding time will facilitate accelerated genetic gain and speed breeding of target crops (Adhikari et al. 2023; Vikas et al. 2021). However, speed breeding necessitates energy-intensive and high-cost growth chamber facilities, which are not readily accessible to maintain optimal growth conditions. Furthermore, speed breeding can subject the growing segregating lines to extreme conditions, wherein selecting specific traits becomes challenging. Thus, in the current study, we have standardized an RGA protocol during the off-season of the wheat crop, specifically in the summer season from April to October. The standardized protocol will facilitate rapid generation advancement of the wheat lines during the off-season in the areas of long photoperiod conditions of sub-tropical regions.

Materials and methods

Plant material

This study utilized HD2932 and HD3086, two bread wheat cultivars, as experimental material. HD2932 has been released for cultivation in the Central and Peninsular agroecological zones, as the late-sown irrigated cultivar demonstrates favorable adaptation (Mallick et al. 2022). HD3086 is known for its high yield potential and is well-suited for cultivation under timely sown irrigated conditions. It has been specifically recommended for cultivation in the North Western Plain Zone (NWPZ) and North Eastern Plain Zone (NEPZ).

RGA conditions

In the present study, the effects of differential seedling densities and irrigation deprivation treatments were tested. For the two differential seedling density assay, the experiment was set up in 500 cm^3 plastic pots for each, one seed/pot and four seeds/pot. The seedlings were grown in the potting mixture of a 2:1:1 ratio of cocopeat, vermiculite, and sand (Rani et al. 2020; Nyamgoud et al. 2022). The plants were grown in a temperature controlled glasshouse chamber under natural daylight conditions. Watering of the plants was given as and when required. However, the irrigation treatment includes water deprivation after 15 and 25 days of anthesis (WD 15 DAA and WD 25 DAA). No supplemental lighting was given at the plant's growth and developmental stage. The temperature of the glasshouse was maintained at a range of 21-22°C to facilitate optimal plant growth (Watson et al. 2018; Samineni et al. 2020). All experiments in the present study were conducted at the Greenhouse, National Phytotron Facility, ICAR-Indian Agricultural Research Institute, New Delhi. As Delhi lies in the country's North-Western Plain Zone (NWPZ), the natural photoperiod ranges from 12 to 14 hours, with sub-tropical conditions prevailing during the off-season of the wheat crop (April-October).

RGA factor 1: Differential seedling density

For differential seedling density assay, the fresh field harvested seeds of HD3086 and HD2932 were taken for sowing. Seeds of both varieties were sown in a 4″ diameter pot, which can hold up to 500 cm^3 of potting mix. The seeds were sown at a rate of one seed and four seeds per pot, respectively, representing eight pots for each variety.

These plantlets were grown in the glasshouse under natural day-length conditions and at a temperature range of 21 to 22°C (Bastos et al. 2020). The pots were regularly supplied with Hoagland solution for irrigation and nutrients (Levine et al. 1999).

RGA factor 2: Irrigation withdrawal

For forced maturity, watering of the pots was stopped after 15 and 25 days of anthesis respectively. In four out of eight pots of both cultivars with lower planting density, irrigation was withdrawn after 15 days of anthesis, while in the other four, irrigation was halted 25 days after anthesis. Similarly, in the pots with higher planting density, watering ceased first in half of the pots after 15 days of anthesis, while in the remaining four pots, it was stopped after 25 days of anthesis. Mature spikes from each plant were harvested 20 days after irrigation withdrawal.

Experimental design, data collection, and analysis

Using a two-way factorial design, we have potted two wheat cultivars in a completely randomized design with four replications. A total of 32 experimental units were established using two factors: i) seedling density treatments (one and four seeds per pot) and ii) irrigation deprivation treatments (15 and 25 days post-anthesis). Phenotypic data for agro-morphological traits such as flowering time (the day after 50% full ear emergence), plant height (cm), number of spikes per plant, flag leaf length (cm), flag leaf width (cm), spike length (cm), number of spikelets per ear, number of seeds per plant, thousand kernel weight (grams), and seed to seed cycle (days) were collected. The mean value of all studied traits was subsequently analyzed for statistical significance using the OPSTAT package (Sheoran et al. 1998).

Germination of harvested seeds

The germination (%) assay was used to evaluate the viability of force-matured seeds (from four seeded pots) that were water-deprived at 15 and 25 days after anthesis in comparison to the control (HD2932 and HD3086 seeds harvested from the field). For this purpose, seeds from all treatment groups (water deprivation at 15 and 25 days post-anthesis) were initially soaked in distilled water overnight (12 hours). Subsequently, the pre-soaked seeds from each treatment group were carefully placed onto germination paper in petri dishes. These seeds underwent chilling treatment (kept at a temperature of 4°C) for 48 to 72 hours in a controlled environment using a freezer (VESTFROST). In contrast, the control seeds received no chilling treatment. Following the chilling treatments, seeds were transferred to a dark environment for 48 hours (Watson et al. 2018). The percentage of germination for each treatment group was thoroughly recorded. Throughout the study, seedlings of each treatment group were maintained in three replicates using a completely randomized design.

Coleoptile and radicle length

The radicle and coleoptile lengths of seedlings from each treatment group were measured to assess their vigor and viability. The data were recorded after seven days of chilling treatment for each group with forced-matured seed and compared with reference controls. Furthermore, the radicle and coleoptile lengths of five seedlings per replicate from each treatment group were measured manually using a graduated ruler (Wei et al. 2022).

Results

Effect of planting density on agro-morphological traits

Growth statistics showing the differential effect of planting density on all agro-morphological traits of wheat varieties, HD2932 and HD3086 are presented in Table 1. The anthesis of plants grown at higher density was significantly earlier than those raised at low density. In HD2932, the flower initiation occurs at 56.3 ± 0.24 days in low-dense plants, whereas it attains significantly earlier in high-dense plants (52.0 ± 0.41 days). Similar results were also observed in HD3086, where flower initiation occurs 3-4 days earlier in high-dense plants (56.5 ± 0.29 days) compared to low-dense plants (60.3 ± 0.24 days). Consequently, the changes in height and spike length of the plants grown in low and high density was significantly different with respect to cultivars. The height of plants, as expected, was more in low-dense plants attributable to lesser/no competition for nutrients and water in single-seeded pots compared to pots with four plants (Fig. 1). Similar observations were also recorded for spike length, wherein plants raised in high density had shorter spike lengths as compared to single-seeded pots.

Other yield-related traits, including the number of spikelets per spike, spikes per plant and seeds per plant, also showed significant variation among varieties and treatments. The number of spikelets per spike (15.4 ± 0.18 and 13.1 ± 0.30) was significantly lower in four-seeded pots as compared to single-seeded pots (17.3 ± 0.16 and 18.3 ± 0.31) in HD2932 and HD3086, respectively. There was only a single spike per plant in a four-seeded pot, while the number of spikes in single-seeded pots varied from 2.1 ± 0.13 and 2.9 ± 0.13, respectively (Fig. 1). Moreover, the number of matured seeds per plant was significantly higher in single-seeded pots (57.6 ± 1.24 and 82.6 ± 1.83) compared to four-seeded pots (19.8 ± 0.73 and 25.8 ± 0.97) in HD2932 and HD3086, respectively. A similar trend was also observed for flag leaf length and flag leaf width in both cultivars (Table 1).

Effect of planting density and irrigation deprivation treatment on agro-morphological traits

The principal aim of the present investigation is to enhance the breeding efficiency of the studied plant by shortening generation time. As reported, factors such as planting

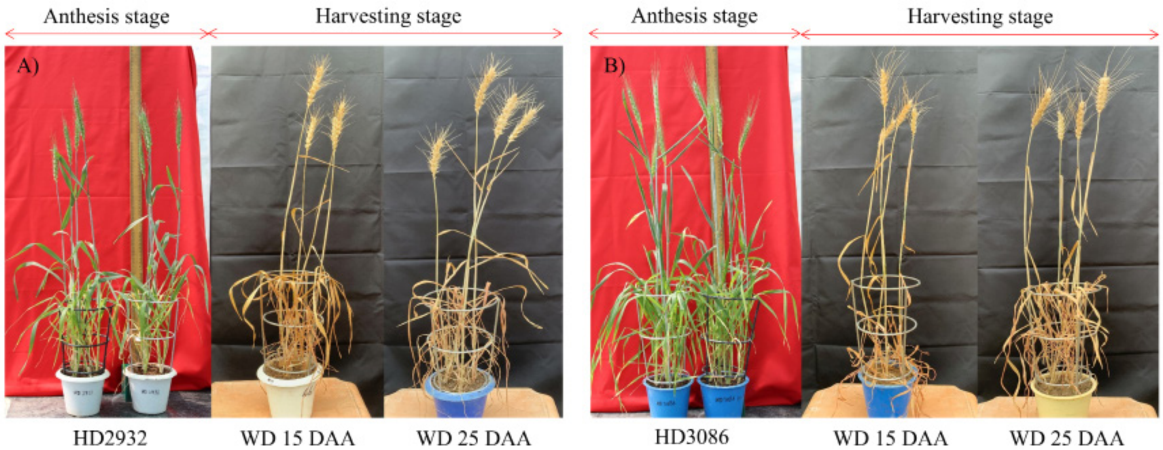

Fig. 1. Experimental design showing the effect of planting density (four seeds per pot) on agro-morphological traits of wheat varieties HD2932 and HD3086 at differential water deprivation treatment after anthesis (WD_DAA)

density, photoperiods, and temperature regimes have notably reduced flowering time in various crops. During the study, we ceased irrigation after 15 and 25 days of flower initiation (anthesis). This led to the forced maturity of both cultivars in each treatment group (four-seeded and single-seeded pots). HD2932 exhibited significantly shorter mean generation time (84.6 ± 0.56 and 89.5 ± 0.64 days) than HD3086 (89.1 ± 0.56 and 93.5 ± 0.64 days) in lower and higher-density treatments, respectively, when irrigation ceased at 15 DAA. Similarly, plants whose irrigation was stopped at 25 DAA have shown extended generation time in both HD2932 (94.8 ± 0.52 and 101.0 ± 0.41 days) and HD3086 (99.6 ± 0.58 days and 105.0 ± 0.41) in four-seeded pots and single-seeded pots, respectively (Table 2).

Moreover, the thousand kernel weights of HD2932 and HD3086 exhibited highly significant variations at WD 15 and 25 DAA in each treatment group (four-seeded and single-seeded pots). At 15 DAA, plants of four-seeded pots (20.3 ± 1.97 and 16.0 ± 0.52 g) exhibited significantly lower thousand kernel weight than single-seeded pots (31.3 ± 1.03 and 29.2 ± 0.20 g) in HD2932 and HD3086, respectively. A similar pattern was observed in each treatment group at WD 25 DAA at

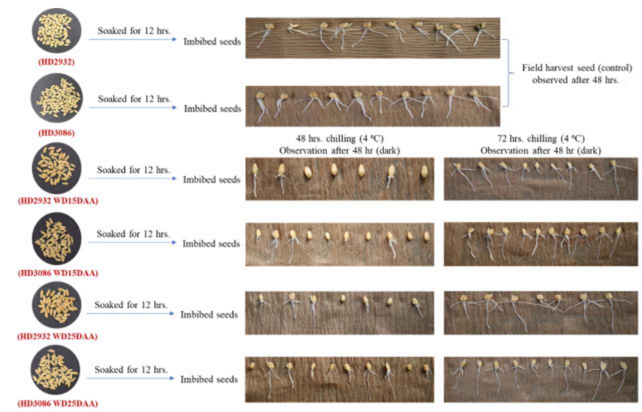

Fig. 2. Effect of differential chilling treatments on percent seed germination of forced-matured seeds of wheat varieties HD2932 and HD3086 in response to differential water deprivation treatments after anthesis (WD_DAA)

differential seedling densities in both varieties (Table 2).

Effect of irrigation deprivation and chilling treatment

Differential times of irrigation withdrawal have also led to changes in grain weight, which resulted in differential germination rates and, consequently, its viability. To measure

Table 1. Growth statistics showing the effect of planting density on different agro-morphological traits of wheat varieties, HD2932 and HD3086

S. No.	Agro-morphological Traits	HD2932		HD3086	
		4 seeds/ pot*	1 seed/ pot#	4 seeds/ pot*	1 seed/ pot#
1	Plant height (cm)	55.6 ± 0.41	62.6 ± 0.84	56.9 ± 0.44	64.3 ± 0.85
2	Flowering time (Days)	52.0 ± 0.41	56.3 ± 0.24	56.5 ± 0.29	60.3 ± 0.24
3	Spike length (cm)	8.2 ± 0.09	8.6 ± 0.13	7.6 ± 0.17	9.6 ± 0.20
4	Number of spikes	1.0 ± 0.00	2.1 ± 0.13	1.0 ± 0.00	2.9 ± 0.13
5	Number of spikelets per spike	15.4 ± 0.18	17.3 ± 0.16	13.1 ± 0.30	18.3 ± 0.31
6	Number of seeds per plant	19.8 ± 0.73	57.6 ± 1.24	25.8 ± 0.97	82.6 ± 1.83
7	Flag leaf length (cm)	11.0 ± 0.20	13.9 ± 0.49	13.9 ± 0.21	15.5 ± 0.26
8	Flag leaf width (cm)	1.0 ± 0.02	1.1 ± 0.02	1.0 ± 0.02	1.1 ± 0.03

*Data taken from 32 plants from 8 pots; # Data taken from 8 plants 8 pots.

Table 2. Growth statistics showing the effect of planting density on different agro-morphological traits of wheat varieties, HD2932 and HD3086

S. No.	Traits	HD2932				HD3086			
		4 seeds/ pot*		1 seed/ pot#		4 seeds/ pot*		1 seed/ pot#	
		WD 15 DAA@	WD 25 DAA@	WD 15 DAA@	WD 25 DAA@	WD 15 DAA@	WD 25 DAA@	WD 15 DAA@	WD 25 DAA@
1	Seed to seed cycle (days)	84.6 ± 0.56	94.8 ± 0.52	89.5 ± 0.64	101.0 ± 0.41	89.1 ± 0.56	99.6 ± 0.58	93.5 ± 0.64	105.0 ± 0.41
2	Thousand kernel weight (grams)	20.3 ± 1.97	34.6 ± 1.87	31.3 ± 1.03	38.0 ± 1.22	16.0 ± 0.52	24.4 ± 0.92	29.2 ± 0.20	35.2 ± 0.69

*Data taken from 16 plants from 4 pots; # Data taken from 4 plants from 4 pots; @ WD__DAA, water deprived __ days after anthesis.

the viability of the matured seeds harvested from plants grown at four seeds per pot, the seeds were germinated by exposing them to two different periods, 48 hours and 72 hours of chilling treatment at 4°C. The seeds exposed to 72 hours of chilling have shown significantly higher percent germination of forced-mature seeds at WD 15 DAA and WD 25 DAA in both varieties compared to control and 48 hrs of chilling treatments (Fig. 2; Table 3). In contrast, HD2932 and HD3086 do not exhibit any significant variations in the shoot and root length upon chilling treatments, either at WD 15 or WD 25 DAA, compared to the control (Fig. 3; Table 3).

Discussion

Innovations in breeding methods and agricultural practices have significantly influenced the global food production. However, rapid demographic growth, changing climatic conditions, a reduction in arable land, and rising concerns about malnutrition have intensified the demand for high-yielding, better-adapted, and more nutritious food crops. Breeding programs must integrate new and innovative tools, technologies and breeding strategies to sustain global food and nutritional security. These integrated breeding tools and techniques will enable scientists and plant breeders to identify superior lines and enhance genetic gain, thereby expediting the variety development and release program. The breeding of advanced and new cultivars often takes seven to nine years to develop genetically stable homozygous lines following the selection and hybridization of parents. Off-season nurseries also provide an opportunity for an additional generation, although a shorter duration is still required.

Recently, successful breeding has been achieved in various field crops by reducing generation time. Approaches that combine increased planting density (Collard et al.

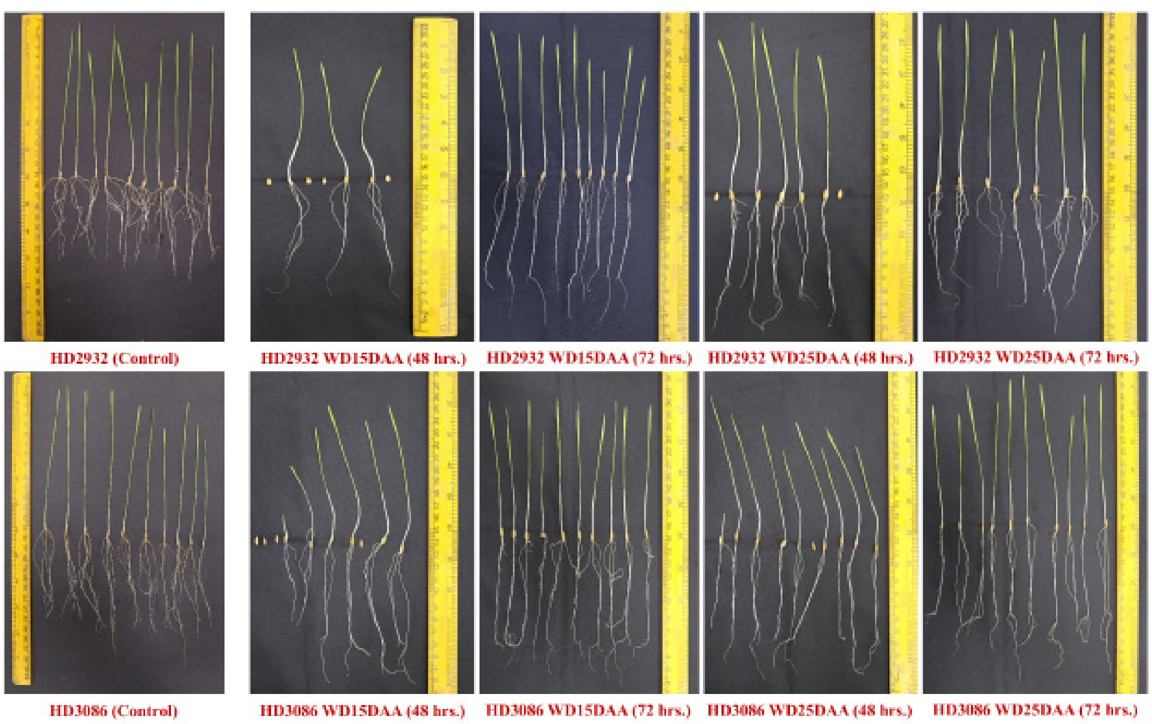

Fig. 3. Effect of differential chilling treatments on shoot length and root length showing the vigour of forced-matured seeds of wheat varieties HD2932 and HD3086 at differential water deprivation treatment after anthesis (WD_DAA)

Table 3. Growth statistics of force matured seeds of wheat varieties HD2932 and HD3086 showing the effect of chilling regime on seedling traits

S. No.		HD2932					HD3086				
	Treatments	Control	WD 15 DAA@		WD 25 DAA@		Control	WD 15 DAA@		WD 25 DAA@	
	Chilling hours (4 °C)	0 hrs.	48 hrs.	72 hrs.	48 hrs.	72 hrs.	0 hrs.	48 hrs.	72 hrs.	48 hrs.	72 hrs.
1	Germination (%)	93.3 ± 3.33	67.3 ± 10.12	93.3 ± 6.67	76.1 ± 6.98	96.7 ± 3.33	90.0 ± 5.78	58.9 ± 4.84	93.3 ± 6.67	76.7 ± 3.33	96.7 ± 3.33
2	Shoot length (cm)	14.5 ± 0.21	11.5 ± 0.30	11.7 ± 0.35	12.6 ± 0.95	13.6 ± 0.37	13.7 ± 0.41	10.9 ± 0.89	11.1 ± 0.47	11.6 ± 0.65	11.9 ± 0.67
3	Root Length (cm)	9.5 ± 0.33	11.3 ± 0.61	11.2 ± 0.65	12.6 ± 0.51	11.7 ± 0.48	9.8 ± 0.23	11.8 ± 0.33	12.3 ± 0.75	13.0 ± 0.36	11.5 ± 1.13

@ WD__DAA, water deprived __ days after anthesis.

2017; Rahman et al. 2019; Kigoni et al. 2023), extended photoperiods, and higher light intensity (O'Connor et al. 2013; Watson et al. 2018; Samineni et al. 2020; Jähne et al. 2020; Kigoni et al. 2023), embryo rescue (Zheng et al. 2013), restricted soil moisture (Zheng et al. 2013; Hussain et al. 2018), regulated temperature (Stetter et al. 2016), higher CO_2 concentration (Tanaka et al. 2016; Nagatoshi et al. 2018), plant hormones (Mobini et al. 2015), and immature seed germination (Saxena et al. 2017; Watson et al. 2018; Jähne et al. 2020) are generally employed for the speed breeding of field crops. Combining these approaches, RGA protocols have been optimized in several crops (Mobini et al. 2015; Liu et al. 2016; Hickey et al. 2017; Watson et al. 2018; Samineni et al. 2020; Jahne et al. 2020; Cha et al. 2022; Kigoni et al. 2023; Kabade et al. 2024). These methods can be both cost-effective and time-saving for fast-breeding off-season crops. The speed breeding protocol proposed by Watson et al. (2018) in wheat employed an extended photoperiod of 22 hours and seven days of moisture stress, and then immature seeds were harvested by the 62nd day after sowing. The generation time under extreme conditions has been significantly reduced with this methodology, but it has increased the need for precise selection using a large number of markers in a large population to identify the best plants in segregating generations or those most similar to recurrent parent in the case of MABB. Moreover, the plants may not display the desired agromorphological traits that the breeder relies on to select the best plants, which restricts the breeder's selection power. Thus, a balanced approach between intense-speed breeding and total reliance on natural conditions can be beneficial in resource-limited breeding programs.

In the present study, we employed a higher planting density to facilitate early flowering and limited water supply to accelerate the maturation phase. The increased planting density (four seeds per pot) resulted in a flowering time that was 3 to 4 days early compared to low density (one seed per pot). Furthermore, the plants were subjected to optimal watering conditions for 15 and 25 days, after which watering was discontinued. Mature seeds were collected following 20 days of water deprivation, and it was observed that the seed-to-seed life cycle demonstrated an accelerated completion. With higher plant density, the seeds were harvested at 89.1 ± 0.56 and 84.6 ± 0.56 days in HD3086 and HD2932, respectively, with irrigation ceasing 15 days after anthesis. Conditions with lower density resulted in a longer duration required for plants to reach maturity. However, no statistically significant difference was observed when irrigation ceased 25 days after anthesis, regardless of whether the plants were raised at high or low density. Therefore, in cultivars that typically take 140 to 150 days to mature under natural field conditions, seeds were harvested in approximately 90 to 100 days under natural day-length condition during summer (at 21–22°C). The earliness observed in high-density planting is generally triggered by moisture stress and plant competition (Wada et al. 2010).

Speed breeding modifies the concentrations of phytohormones in developing embryos, thus speeding up the growth and development of seedlings (Kigoni et al. 2023). The seeds harvested exhibited low germination rates, potentially due to high abscisic acid (ABA) levels in the embryo. For successful germination, a proper balance between ABA and gibberellic acid (GA) is essential (Shu et al. 2016). The elevated ABA levels in the embryo may be associated with water stress experienced during maturation or the embryo's premature harvesting before reaching full maturity. Seed germination under these conditions can be enhanced through the external application of GA or by chilling the seeds (Tuttle et al. 2015). These methods optimize the ABA to GA ratio, promoting successful seed germination.

Consequently, the collected seeds that underwent chilling treatments lasting for 48 and 72 hours, following 12 hours of water imbibition performed well. The germination percentages of HD2932 and HD3086 were 67.3 ± 10.12 and 58.9 ± 4.84%, respectively, after a 48-hour chilling treatment when watering ceased at 15 DAA. In contrast, both treatment groups (WD 15 DAA and WD 25 DAA) exhibited improved seed germination by over 90% due to the 72-hour chilling treatment. The results indicated that extending the chilling duration to 72 hours could significantly enhance seed germination rates. Furthermore, an optimal number of segregating lines utilized for selection and hybridization is crucial for the rapid generation advancement of field crops. Accordingly, the proposed methodology produces the optimal number of seeds, which can be achieved by increasing tillers and reducing planting density. In the study, an average of 19.8 ± 0.73 and 25.8 ± 0.97 seeds were harvested respectively from HD2932 and HD3086, at higher densities while 57.6 ± 1.24 and 82.6 ± 1.83 seeds can be harvested respectively from single plants in a pot. The thousand kernel weight (TKW) observed from plants grown at different planting densities can be attributed to a longer duration that enhances the filling of photosynthates in the flag leaf. The differential accumulation of photosynthates also increases the length and width of the flag leaf (Watson et al. 2018; Kigoni et al. 2023).

Therefore, the current protocol can be utilized for rapidly advancing and selecting plants in the segregating generations by cultivating individual plants in pots, as it allows for the adequate expression of agronomic traits, providing the opportunity for targeted selection of desirable traits. The proposed method can be adopted in a pedigree breeding scheme, in which the first two generations of raising parents, including hybridization, as well as raising F_1 plants, can be undertaken off-season. Meanwhile, F_2 can be grown in the main season in the targeted environment. In the second year, the two generations, namely F_3 and F_4, can be rapidly advanced in the off-season as per the protocol, and in the main seasons, the best progeny rows can be selected under natural field conditions. Hence, the aforementioned approach can effectively expedite the breeding of lines used for varietal development. Additionally, the protocol can be integrated into a backcross breeding program. From our lab, Raj et al. (2024) incorporated the proposed protocol and transferred the *Lr52* leaf rust resistance gene into the background of HD3086 within 2.5 years. We have taken the crucial generations, namely BC_1F_1 and BC_2F_3, during the main seasons, while the remaining generations were rapidly advanced in the off-season. Adhering to this methodology makes it more feasible to complete a breeding cycle (approximately 90-95 days) without the necessity for additional artificial light. In conclusion, the proposed rapid generation advancement (RGA) protocol will facilitate the production of two generations of plants during the off-season of summer, which can be effectively integrated by taking a crop in the main season and can be used to develop wheat cultivars at a faster pace.

Authors' contribution

Conceptualization of research (SKJ, NM, AKMB, LS); Designing of the experiments (SKJ, NM, AKMB, LS); Contribution of experimental materials (SKJ, NM, RK, MN); Execution of field/lab experiments and data collection (NR, HS, RB, SB, UK, AKC, PA); Analysis of data and interpretation (NR, AKC, PA, SKJ, LS); Preparation of the manuscript (NR, AKC, SKJ, NM).

Acknowledgments

The first author is grateful to the ICAR PG Scholarship for financial assistance during his studies at The Graduate School, ICAR-IARI, New Delhi. The authors also thank the National Phytotron Facilities, ICAR-IARI, New Delhi, for facilitating the experiments conducted during the study. The corresponding author expresses his gratitude to ICAR, Govt. of India sponsored project 'CRP on hybrid technology' entitled "Hybrid technology for higher productivity in selected field and horticultural crop (Wheat)" (Project file no.- ICAR/IARI/CRP HT/2015/1912 Dated: 18/05/2015).

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