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Development of high yielding and stress resilient post-rainy season sorghum cultivars using a multi-parent crossing approach

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Modern agriculture, based on biparental crop varieties have contributed tremendously to the world's food supply. However, the strategy is also being challenged due to stagnation in yield growth, climate change, susceptibility to biotic and abiotic stresses etc. Biparental crossing, the conventional cereal breeding approach, is inherently limited in its ability to fully harness the rich genetic diversity available within a crop species. This limitation stems from the restricted number of parental lines involved, which restricts the pool of desirable traits that can be combined. In contrast, cutting-edge multi-parental crossing strategies possess immense potential for generating superior trait combinations by tapping into a vastly broader genetic pool. However, despite the several advantages of this approach, its full potential has not been adequately exploited. The existing research on the development of multi-parent advanced generation inter-cross (MAGIC) populations in crops such as rice, maize, and sorghum has primarily focused on the populations themselves, lacking robust demonstrations of the potential advantages of this approach over biparental crossing in terms of developing superior crop varieties. This study aimed to develop post-rainy season sorghum genotypes with enhanced yield potential and improved tolerance to drought, shoot fly, and charcoal rot through the utilization and demonstration of a multi-parent crossing approach. 17 founder lines were utilized to generate four 8-way crosses. The performance of the resulting progeny was systematically evaluated across multiple locations. The results revealed that the 8-way cross-derived lines exhibited remarkable superiority in both grain and stover yields, outperforming not only the 2-way and 4-way cross derivatives but also their founder parents. Notably, the 8-way cross-derived lines demonstrated substantial yield advantages of over 70% and 30% in grain and stover production, respectively, compared to the bi-parent crosses. These lines also displayed enhanced drought tolerance and improved resistance against key insect pests and diseases. Specifically, two 8-way cross-derived lines, S22086RV and S22085RV, significantly outperformed the national check cultivar CSV 29R, with nearly 70% and 60% higher grain yields, and over 30% and 15% greater stover yields, respectively. Importantly, these high-performing lines also exhibited exceptional drought stress tolerance, characterized by high transpiration rate, transpiration efficiency, shoot biomass, harvest index, and grain yield coupled with low total water use, as well as resistance against shoot fly (<15% dead hearts) and charcoal rot (<10 charcoal rot index). These versatile, stress-resilient lines hold immense promise as valuable genetic resources to drive further crop improvement and the development of superior post-rainy sorghum varieties. This innovative breeding strategy demonstrates significant potential for transforming post-rainy sorghum cultivation, particularly in contexts constrained by limited phenotypic diversity that impedes progress.

Keywords Sorghum, Post-rainy season, Multi-parent crossing, MAGIC, Drought tolerance, Shoot fly resistance, Charcoal rot resistance

Abbreviations

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MAGIC	Multi-parent advanced generation inter-cross
MPCD	Multi-parent cross derivative
WUE	Water use efficiency
TR	Transpiration rate
TE	Transpiration efficiency
TWU	Total water use
HI	Harvest index
CRI	Charcoal rot index
DH	Dead hearts

Sorghum, a versatile cereal grain, has gained significant attention in recent years due to its resilience, nutritional value, and potential as a food and fodder crop. Sorghum serves as a staple food for over 500 million resource-constrained individuals residing in approximately 30 countries located in subtropical and semi-arid regions[1]. Globally, sorghum is grown on 40.82 million hectares, producing 61.49 million tons at an average of 1,506 kg/ha[2]. In Eastern Africa, it is the second most widely grown cereal after maize, with around 7 million hectares annually. Sorghum is also a major crop in Ethiopia, grown at various elevations[3]. India ranks first among sorghum-producing countries in terms of area, but is fourth behind the USA in total production. In India, sorghum is the second most important dryland crop after pearl millet, with a productivity of 849 kg/ha and a total production of 3.47 million metric tons from 4.09 million hectares[4].

In India, two distinct adaptive types of sorghum are cultivated—rainy season (kharif) and post-rainy season (rabi) sorghum. The rainy season sorghum is primarily grown for non-food purposes, such as animal feed, fodder, and industrial applications, while the post-rainy season sorghum is almost entirely consumed as a food grain by humans[5]. These two sorghum ecotypes have adapted to the differing climatic conditions and growing seasons prevalent in the country, with the rainy season sorghum thriving during the monsoon months and the post-rainy season sorghum capitalizing on the residual soil moisture after the rains have subsided. In India, the majority of sorghum cultivation, around 80%, takes place during the post-rainy season, while the remaining 20% is in the rainy season. Post-rainy season sorghum mostly preferred as a dual-purpose crop due its excellent grain and fodder quality and is a crucial source of sustenance and income for millions of households in India[6]. The productivity of post-rainy season sorghum is influenced by a complex interplay of environmental, agronomic, and socioeconomic factors. One major factor contributing to low productivity is the unpredictable and variable nature of rainfall patterns during this season. Rainfed farming systems in India often face frequent droughts and high variability in rainfall, which can significantly impact crop growth and yields. Terminal drought, a widespread problem, can lead to yield losses of up to 30–40% during the post-rainy season[7]. The shoot fly is a significant biotic constraint that substantially impairs sorghum productivity and this insect pest can inflict severe yield losses by causing damage to the crop at the seedling stage, resulting in reduced plant stand and compromised growth. Extensive shoot fly infestations have the potential to lead to crop losses as high as 80–100%[8]. Charcoal rot, a disease that affects the sorghum crop during the post-flowering stage, is also a significant constraint that can contribute to yield losses of up to 40–50%. This fungal disease can impair grain development and reduce the overall productivity of the crop. Additionally, the quality and nutritional value of the sorghum fodder may also be adversely affected by charcoal rot, further compromising the crop's utility as an important livestock feed[9].

Unlike rainy sorghum, which is dominated by hybrids, post-rainy sorghum is primarily cultivated with varieties, particularly landrace selections. A significant portion of the area is still occupied by the landrace variety M 35-1, developed over seven and a half decades ago. Post-rainy sorghum research did not receive substantial focus until the 1990s, and the varieties or hybrids bred and released since then have struggled to match the yield and quality of M 35-1. Recently, some progress has been made with the introduction of varieties adapted to specific soil depths, such as shallow, medium, and deep soils[10]. However, the productivity of post-rainy sorghum remains low, constrained by biotic and abiotic factors. Over the past 25 years, several programs in India have attempted to improve varieties or hybrid parents through pedigree breeding approaches. Although the area under rabi sorghum has remained fairly consistent over the years, the progress in productivity has been much slower compared to rainy sorghum[11]. This low rate of productivity necessitates a change in production strategy, including breeding approaches. Breeding for monogenic varieties, focusing on a single specific trait or high grain yield alone, has not been particularly rewarding in post-rainy sorghum. Instead, a more comprehensive approach that considers multiple traits, such as drought tolerance, disease resistance, and high biomass production, along with grain yield, is crucial to develop resilient and high-performing post-rainy sorghum varieties[6,12–14]. The resource-constrained sorghum cultivating farmers generally favor varieties that possess key traits, such as dual-purpose capabilities, bold seeds, medium maturity, resistance to lodging, drought tolerance, and protection against insect pests and diseases. Therefore, there is a need to plan and devise strategic research programs in rabi sorghum to break the existing yield plateau and develop multi-genotype varieties that combine the most desirable traits[2]. Additionally, exploring strategies to broaden the genetic base of sorghum breeding programs, such as integrating diverse germplasm sources, employing advanced breeding techniques, and establishing collaborative networks to share genetic resources and knowledge, could be crucial in developing new cultivars that combine the preferred traits while also increasing the overall productivity and resilience of post-rainy season sorghum production[15].

The limited genetic diversity within breeding lines can further restrict the genetic improvements attainable for post-rainy sorghum. Several studies have indicated that the pure-line selection among local or popular sorghum varieties led to the development of many improved cultivars. However, the genetic gains achieved through this approach have been modest, particularly in the case of post-rainy sorghum[16,17]. Modern agriculture, based on biparental crop varieties have contributed tremendously to the world's food supply. However, the strategy is also being challenged due to stagnation in yield growth, climate change, susceptibility to biotic and abiotic stresses etc.[18]. Conventional methods and genetic mapping populations used in plant breeding are typically based on biparental crosses, such as recombinant inbred lines, F_2 populations, doubled haploids, and backcrosses[19]. The prevailing breeding approaches in crops like sorghum primarily rely on biparental crosses to generate the variations exploited by breeders. While these biparental populations have been widely employed, they often exhibit relatively low genetic diversity due to the limited recombination rates, which can result in a high probability for the parental lines to carry the same alleles at a given locus[20]. Biparental populations typically have only one opportunity for genetic recombination, with each crossing generation estimated to have around 34 potential recombination points. The restricted number of parental lines involved may constrain the overall genetic diversity and the potential for discovering novel traits[21]. To address this issue and expand the genetic diversity available for genetic dissection and trait improvement, multiparent genetic populations, such as multiparent advanced generation inter-cross and nested association mapping populations, have been increasingly established and utilized in various crop species[22]. To increase genetic variation in breeding populations, breeders have attempted to make multiple crosses. However, the extensive use of these multi-parent crosses has been limited by technical challenges. More complex crossing schemes involving 6-way, 8-way, or diallel selective mating, though proposed long ago, have rarely been utilized in sorghum breeding programs, despite their potential for self-pollinated crops[23].

Multi-parental crossing, also referred to as synthetic or composite crossing, facilitates the incorporation of a broader range of genetic diversity, potentially leading to the development of more climate-resilient and high-yielding cultivars[24]. The use of multi-parent crosses has a long-standing history in conventional plant breeding, particularly in outcrossing crops where such crosses are relatively straightforward[25,26]. In self-pollinated species like sorghum, multi-parent crosses became more common in the 1960s following the discovery of genetic male sterility systems, which facilitated the adoption of recurrent selection[27,28]. The utilization of MAGIC populations enables the targeting of a broader range of diverse traits for genetic analysis compared to traditional biparental recombinant inbred line populations, contingent on the contrasting parental lines involved in the MAGIC population's construction. Multiparent populations like MAGIC have increased the likelihood of identifying quantitative trait loci that exhibit polymorphism across multiple parental lines, thereby enhancing the mapping resolution and statistical power for QTL detection[29]. As a multiparent panel, MAGIC populations are particularly well-suited to identify favorable alleles from diverse parental lines, which can be leveraged to dissect the genetic variation more precisely for complex traits. Furthermore, MAGIC populations allow for the inclusion of greater genetic diversity, smaller haplotype blocks, higher recombination rates, and improved QTL mapping resolution compared to typical biparental populations. Despite the growing body of research aimed at understanding the genetic basis of various traits in sorghum, substantial gaps remain in our comprehensive understanding of the genetic architecture of many important agronomic and quality traits in this crucial cereal crop[30].

The emergence of multi-parent advanced generation intercross populations has provided innovative next-generation mapping resources to address the key limitations of conventional biparental and association mapping populations. MAGIC and MAGIC-like populations have now been developed across a range of plant and crop species, including model organisms like Arabidopsis as well as major crops like wheat, rice, and maize. The pioneering mouse collaborative cross was the first MAGIC population developed, paving the way for the application of this powerful quantitative genetics approach to mapping complex traits in crops[31]. Since then, several MAGIC populations have been developed and used for quantitative trait locus discoveries in other major field crops including wheat[32,33], rice[34], tomato[35], faba bean[36], maize[37,38], barley[39], soybean[18] and sorghum[40]. This provided a diverse genetic background to interrogate the complex genetic architecture underlying important agronomic and quality traits in this drought-resilient cereal crop. More recently, a first structured non-random mating-MAGIC population was created in grain sorghum by strategically inter-crossing 4 founders that individually represent the major botanical races of sorghum—kafir, guinea, durra, and caudatum[41]. This targeted design is expected to capture greater genetic diversity and enable more effective QTL mapping and breeding applications. The first high-density genetic linkage map of an eight-parent MAGIC population has now been reported in soybean, demonstrating the utility of these complex populations for advanced genetic analysis and improvement of important legume crops as well[42].

Despite the several advantages of this approach, its full potential has not been adequately exploited. The existing research on the development of MAGIC populations in crops such as rice, maize, soybean and sorghum has primarily focused on the populations themselves, lacking robust demonstrations of the potential advantages of this approach over biparental crossing in terms of developing superior crop varieties. As an often-cross-pollinated crop, sorghum offers excellent opportunities for manual emasculation and crossing, allowing numerous parents to contribute desirable features to the final product. The application of multiparent crossing strategies in post-rainy season sorghum breeding can significantly enhance the genetic gain per unit of time and resources invested, as it has the potential to break the yield plateau that has been observed in sorghum breeding programs. In this context, the present study aimed to: (i) employ and demonstrate a multiparent crossing approach to combine desirable multiple traits from a set of eight founder lines adapted to the post-rainy ecosystem, (ii) evaluate multi-parent cross derived lines for key agronomic traits, drought, shoot fly and charcoal rot tolerance, and (iii) identify the dual purpose high yielding lines with improved tolerance to drought, shoot fly and charcoal rot suited to post-rainy season cultivation. The study sought to leverage the power of multi-parent crossing to generate novel genetic variations and elite breeding lines that could address the key challenges faced in sustainable post-rainy sorghum improvement.

Material and methods
Plant material
To develop the multi-parent crosses and their advanced generation derivatives, a carefully curated set of 17 elite post-rainy season sorghum lines were strategically selected as the founder parents. These genetically diverse parental lines were selected based on their diverse phenotypic performance for important agronomic, and biotic and abiotic stress related traits. The chosen parents represented a diverse array, including high-yielding grain and stover types, disease and insect tolerant types, as well as geographically distinct, natural, or semi-natural populations (Table 1). Since the post-rainy season genotypes and their growing conditions are entirely different from those of the rainy season genotypes, great care has been taken in selecting the founder lines possessing only post-rainy season adaptive traits, in order to avoid the genetic contamination and undesirable linkage drags that could otherwise occur.

Breeding strategy
A multi-parent crossing scheme representing a multi-stage hybridization followed by pedigree selection was employed to develop multi-parent crosses. This comprehensive approach involved the inter-crossing in the form of straight /pair-wise crosses, consisting of a set of eight parents separately from among the 17 founder lines. Four such multi-parent crosses were developed in this study. The multi-parent crossing scheme consisted of three rounds of hybridization. In the first round, a set of eight parents were inter-crossed in a pairwise manner to generate four 2-way crosses. In the second round, these 2-way cross F_1 plants were inter-crossed to produce two 4-way crosses. Ten F_1 plants with good panicle emergence and an intermediate expression were chosen for emasculation and pollination to generate the 4-way crosses. In the third and final round, the two 4-way crosses were again inter-crossed, with the individuals of one 4-way cross serving as the female group and the individuals of the other 4-way cross as the male group. In this case, five of the best segregating plants exhibiting superior plant and panicle characteristics were selected from each progeny of the female 4-way cross. These selected female plants were then pollinated by their corresponding five best plants chosen from each progeny of the male group. This meticulous inter-crossing between the individuals of these two groups produced 50

Founder lines	Pedigree	Developed by	Duration (days)	Distinctive traits
CSV 14R	[M35-1 × (CS2947 × CS2644) × M35-1]	DSR-Hyderabad, Telangana	Medium (125–130)	Suitable for medium to deep soils of all rabi growing areas of the country. Pearly white, bold grain, tolerant to shoot fly and charcoal rot
Solapur Dagdi	Popular local landrace	Grown widely in the southwestern region of Maharashtra	Medium (120–125)	Drought tolerant, compact panicles, excellent grain quality
CRS 4	Elite breeding line	CRS, Solapur, Maharashtra	Early (100–115)	Drought tolerant, superior panicle characters
Phule Revati	CSV 216 × SPV 1502	MPKV-Rahuri, Maharashtra	Medium (120–125)	Suitable for deep soils and under irrigated conditions. Pearly white round and very bold grain, tolerant to shoot fly and charcoal rot
CSV 22	SPV 12359 × RSP 2	MPKV-Rahuri, Maharashtra	Medium (116–120)	High yielding, long panicles, tolerant to drought and charcoal rot
Phule Chitra	SPV655 × RSLG 112	MPKV-Rahuri, Maharashtra	Early (110–115)	Suitable for medium soils. Plumpy, lustrous and medium bold grain, good grain and fodder quality, tolerant to shootfly, charcoal rot, drought and non-lodging
PKV Kranti	SPV-1201 × Ringni	VNMKV-Parbhani, Maharashtra	Medium (125–130)	Suitable for medium to deep soils and irrigated. Pearly white round and very bold grain, tolerant to shoot fly
Phule Suchitra	SPV 1359 × SPV 1502	MPKV-Rahuri, Maharashtra	Late (130–135)	Suitable for medium soils. Good roti quality characters
CSV-216R	Selection from Dhulia landrace	MPKV-Rahuri, Maharashtra	Medium (120–125)	Suitable for medium to deep soils in rainfed and irrigated areas, Pearly white round, lustrous and medium bold grain. Tolerant to shoot fly and drought
Parbhani Moti	Selection from GD 31-4-2-3	VNMKV-Parbhani, Maharashtra	Late (130–135)	Suitable for medium soils in rainfed areas. Pearly white lustrous and bold round grain. Tolerant to shoot fly and charcoal rot. Responsive to fertilizers
CRS 20	Elite breeding line	CRS-Solapur, Maharashtra	Late (130–135)	Drought tolerant line with better panicle features
DSV 5	Selection from Natte maldandi variety of Gulbarga locality	MARS-Dharwad, Karnataka	Medium (120–125)	Suitable for deep soils, transitional and irrigated zones in Karnataka. Pearly white round and very bold grain, tolerant to shoot fly
Selection 3	Selection from Bidar rabi local	MPKV-Rahuri, Maharashtra	Medium (125–130)	Dwarf, drought tolerant variety
DSV 4	E 36-1 × SPV 86	MARS-Dharwad, Karnataka	Medium (115–120)	Suitable for dry zones in Karnataka. Creamy bold, round grain flat at one side and tolerant to diseases
CSV 29R (NC)	(CSV 216 × DSV 5) × CSV 216R	RARS-Vijayapura, Karnataka	Medium (125–130)	National check for rabi grain and stover yields, tolerant to charcoal rot and rust
CSV 26R (NC)	SPV 655 × SPV 1538	CRS-Solapur, Maharashtra	Early (112–115)	Suitable for shallow soils, tolerant to shoot fly, stem borer, charcoal rot and tolerant to drought. Medium bold grain with good fodder quality
M 35-1 (PLC)	Selection from maldandi landrace	ARS-Mohol, Maharashtra	Medium (125–130)	Popular among the rabi varieties, Insect tolerant, excellent roti and stover quality

Table 1. Detailed information on the pedigree and distinctive characteristic features of the 17 founder lines that were used as parents in the development of the four multi-parent crosses described in this study. The table includes key details about the genetic backgrounds and specific traits of these founder lines, which were strategically selected to enable the combination of desirable characteristics in the subsequent multi-parent crossing program.

intermated F_1 plants. The resulting 8-way cross F_1 plants were then self-pollinated to generate a variable F_2 segregating population. The F_2 plants were space-planted at a minimum population size of 5000 individuals, and 500 individual plant selections at 10% selection intensity were advanced for the realization of transgressive segregants. Approximately 200 promising F3 progenies were advanced to the F_6/F_7 generation to attain sufficient homozygosity, with stringent positive phenotypic selection applied during advancement to retain the most promising progenies. The pedigree method was employed for advancing the generations. The corresponding 2-way and 4-way cross F_1 plants were also simultaneously selfed, and the resulting F_2 populations were further advanced in the same manner as the 8-way cross derivatives. The schematic representation of the multi-parent crossing strategy is depicted in Fig. 1**,** and the details of the 2-way, 4-way, and 8-way crosses developed are provided in Table 2.

The crossing work was carried out at the well-maintained research field of the ICAR-Indian Institute of Millets Research, regional station, Solapur. Experimental crossing blocks were established during the post-rainy season of 2015 in the field, and in pots during the subsequent summer and kharif seasons of 2016 to synthesize the desired 8-way crosses in the shortest possible timeframe. Hybridization between the lines was accomplished through manual emasculation followed by controlled pollination[43]. To achieve adequate synchronization among the founder parents, including 2-way and 4-way crosses, and to enable the production of a sufficient number of crossed plants, three staggered sowing dates were implemented at intervals of 7 days. This approach facilitated the accelerated recombination breeding process within a relatively short timeframe, which was crucial for generating the desired multi-parent crosses in a timely manner.

Field experimentation

Field design and station evaluation trials

Comprehensive field evaluation trials were conducted over two consecutive post-rainy seasons, from 2022 to 2023, at the rainfed research farm of the ICAR-Indian Institute of Millets Research, regional station, Solapur, Maharashtra, India (Latitude: 17° 40′ N, Longitude: 75° 54′ E, Altitude: 473 m above sea level). The 45 genotypes, which included sixteen 2-way, eight 4-way, and four 8-way cross derivatives, along with their seventeen parental

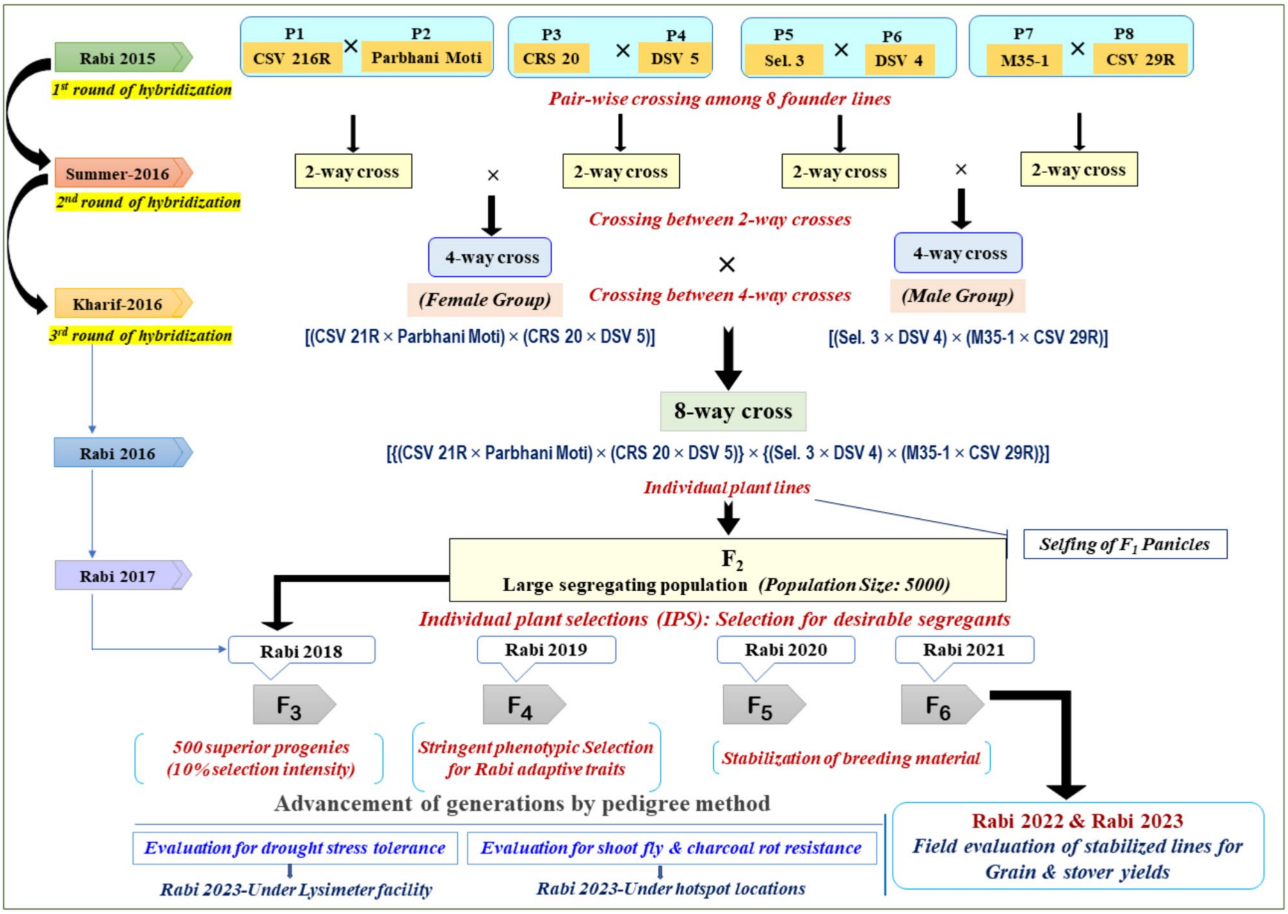


Fig. 1. Schematic illustration of the multi-parent crossing approach employed in this study to generate four multi-parent crosses. The diagram depicts the specific scheme for the development of the 8-way cross-3, involving a multi-stage hybridization process and subsequent pedigree selection. The terms "Rabi" and "kharif" are used to represent the post-rainy and rainy seasons, respectively. The founder lines involved in this crossing approach are represented by P1 to P8.

Type of cross	Identity	Pedigree
2-way cross		
2-way cross-1	S22060RV	CSV 14R × Solapur Dagadi
2-way cross-2	S22061RV	CRS 4 × Phule Revati
2-way cross-3	S22062RV	M 35–1 × CSV 22
2-way cross-4	S22063RV	Phule Chitra × CSV 22
2-way cross-5	S22064RV	PKV Kranti × Solapur Dagadi
2-way cross-6	S22065RV	Phule Suchitra × CSV 14R
2-way cross-7	S22066RV	M 35–1 × CSV 29R
2-way cross-8	S22067RV	CSV 216R × CRS 4
2-way cross-9	S22068RV	M35-1 × CSV 29R
2-way cross-10	S22069RV	Parbhani Moti × CRS 20
2-way cross-11	S22070RV	DSV 5 × Selection 3
2-way cross-12	S22071RV	CSV 216R × CRS4
2-way cross-13	S22072RV	CSV 14R × DSV 4
2-way cross-14	S22073RV	CSV 26 × PKV Kranti
2-way cross-15	S22074RV	CSV 29R × PKV Kranti
2-way cross-16	S22075RV	Parbhani Moti × DSV 5
4-way cross		
4-way cross-1	S22076RV	(CSV 14R × Solapur Dagadi) × (CRS 4 × Phule Revati)
4-way cross-2	S22077RV	(M 35–1 × CSV 22) × (Phule Chitra × CSV 22)
4-way cross-3	S22078RV	(PKV Kranti × Solapur Dagadi) × (Phule Suchitra × CSV 14R)
4-way cross-4	S22079RV	(M 35–1 × CSV 29R) × (CSV 216R × CRS 4)
4-way cross-5	S22080RV	(M35-1 × CSV 29R) × (Parbhani Moti × CRS 20)
4-way cross-6	S22081RV	(DSV 5 × Selection 3) × (CSV 216R × CRS4)
4-way cross-7	S22082RV	(CSV 14R × DSV 4) × (CSV 26 × PKV Kranti)
4-way cross-8	S22083RV	(CSV 29R × PKV Kranti) × (Parbhani Moti × DSV 5)
8-way cross		
8-way cross-1	S22084RV	[(CSV 14R × Solapur Dagadi) × (CRS 4 × Phule Revati)] × [(M 35-1 × CSV 22) × (Phule Chitra × CSV 22)]
8-way cross-2	S22085RV	[(PKV Kranti × Solapur Dagadi) × (Phule Suchitra × CSV 14R)] × [(M 35-1 × CSV 29R) × (CSV 216R × CRS 4)]
8-way cross-3	S22086RV	[(M35-1 × CSV 29R) × (Parbhani Moti × CRS 20)] × [(DSV 5 × Selection 3) × (CSV 216R × CRS4)]
8-way cross-4	S22087RV	[(CSV 14R × DSV 4) × (CSV 26 × PKV Kranti)] × [(CSV 29R × PKV Kranti) × (Parbhani Moti × DSV 5)]

Table 2. The details of the 2-way, 4-way, and 8-way crosses that were generated and the cross combinations that were developed through the multi-parent crossing approach employed in this study. The table includes information on the specific founder lines involved in each type of cross, as well as the resulting cross combinations that were produced.

lines, were planted in an alpha lattice design[44] with three replications. Each entry was grown in a large plot size of 5 m × 0.45 m × 10 rows (22.5 m^2) to facilitate a thorough field evaluation. The entries were fitted into five incomplete blocks, with nine plots per block in each replication. To ensure proper planting, the seeds for each entry were equally distributed into seed packets that were designated with randomized plot numbers as per the field layout. Timely crop management practices were followed to ensure optimal plant growth and development during the entire crop season. Supplementary irrigation was provided as and when required to avoid any moisture stress, especially during the critical stages of crop growth.

Traits evaluation
Data were collected on key phenotypic traits according to the Standard Key Descriptor Lists for sorghum characterization[45]. These included days to 50% flowering (DFF; days) recorded as the number of days from sowing until approximately 50% of the plants reached mid-flowering, days to maturity (DM; days) determined as the number of days from sowing to when the black layer forms a visible dark spot at the base of the kernels, indicating harvestable maturity, plant height (PH; cm) measured from the ground to the tip of the panicle at maturity, panicle exertion (PE; cm) measured from flag leaf to the base of panicle inflorescence, peduncle length (Ped. L; cm), measured from the base of the first node where the sheath of the flag leaf is attached to the bottom of the panicle, panicle length (PL; cm) measured from the lower panicle branch to the tip of the panicle at maturity, panicle width (PW; cm), was taken as the maximum width of the panicle in its natural position, panicle yield (PY; kg/ha) measured as the weight of the total dry panicles in a plot before threshing, grain yield (GY; kg/ha) measured as the mean weight of the grains obtained from the total panicles after threshing, stover yield (SY; kg/ha) measured as the mean weight of the total dry fodder of the plants tied in a bundle in a plot, and 100 grain weight (TW; gm) measured by weighing 100 grains at 12% moisture content.

Screening for drought tolerance using lysimeter

The same set of 45 genotypes, which included sixteen 2-way, eight 4-way, and four 8-way cross derivatives, along with their seventeen parental lines, were screened under a lysimeter facility at the ICAR-Indian Institute of Millets Research (IIMR), Hyderabad. The lysimeter method (long PVC tubes of 2.0 m length and 25 cm diameter) enabled the monitoring of plant water use and biomass accumulation (both vegetative and grain) from the early growth stages to maturity. In each cylinder, eight seeds of each genotype were sown and then thinned to two seedlings per cylinder at 14 days after sowing (DAS), and further thinned to one plant per cylinder at 21 days after sowing. All plants were maintained under fully irrigated conditions until a specific phenological phase (28 DAS). Drought was imposed by ceasing irrigation after complete soil saturation in the cylinders. The drought-stressed (DS) treatment received no water from 28 days after sowing until maturity, except for 2 L added to all cylinders at 73 days after sowing (beginning of grain filling), while the well-watered (WW) treatment was irrigated regularly. After regular irrigation, the cylinders were covered with a 2-cm layer of low-density polyethylene beads to prevent soil evaporation. The soil moisture status in the top 15 and 30 cm soil depth was monitored in both treatments on the day of sowing and subsequently at 15–20-day intervals until maturity using the gravimetric method.

Key water use efficiency traits, such as transpiration efficiency (TE), transpiration rate (TR), and total water use (TWU), were calculated by weighing the cylinders. TE is a measure of how much dry matter a plant produces per unit of water it transpires, i.e., the ratio of plant biomass to water lost through transpiration. TR is the rate at which water evaporates from a plant's leaves into the air. TWU of a plant is the amount of water lost through transpiration plus the amount of water evaporated from the soil. This process is called evapotranspiration, which is the combined loss of water from the soil surface (evaporation) and from the plant (transpiration).

The cylinders were weighed for the first time at 30 days after sowing, and then this process was repeated every 2 weeks thereafter. This provided a total of five weight measurements until harvest for the drought-stressed plants, and six weight measurements for the well-watered plants. The initial weighing at 30 days after sowing allowed for the determination of the field capacity weight of each cylinder. Transpiration was calculated at approximately 2-weekly intervals between 31 days after sowing (the time at which weighing started) and plant maturity. To determine the daily transpiration values for each plant, the transpiration for each time interval between weighings was divided by the number of days in that interval. The pre-anthesis transpiration was calculated as the sum of the daily transpiration values up until the point of anthesis, plus the estimated 1.5 L of water used by all genotypes during the first 28 days after sowing.

The water use after the flowering stage (post-anthesis) was calculated by summing the daily transpiration values from the flowering stage until plant maturity. The plants were harvested over a 4-day period, and the aboveground plant biomass was measured after drying the samples in a forced-air oven at 70 °C for 3 days. The panicles were then threshed to determine the grain yield. The harvest index (HI) was calculated as the ratio of grain yield to the total aboveground biomass. The transpiration efficiency was calculated as the ratio of the total aboveground biomass to the sum of transpiration values between 30 days after sowing and maturity. The contribution of these traits to grain yield under terminal drought stress was calculated using the formula: Yield = TWU × TE × HI[46].

Lysimeter growing conditions

The study used mini-lysimeters—polyvinyl chloride tubes with a 25 cm diameter and 2.0 m length—to grow plants under open field conditions. A rain-out shelter was used to cover the lysimeters during rainfall events (Fig. 2). The soil was an Alfisol, a neutral sandy clay loam to clay type. At the base of each cylinder, a PVC plate was positioned 3 cm above the bottom to hold the soil in place while allowing drainage. The soil was obtained from the IIMR farm and had aggregates smaller than 1 cm. It was added in three 40-kg increments, with watering after each to ensure proper settling. The soil had a field capacity of approximately 20% for water retention, so 8 L of water were added to each 40-kg increment. After the three increments, an additional 15 kg of dry soil was introduced, followed by 3 L of water. The cylinders were then filled to within 5 cm of the top with air-dry soil to ensure uniformity, with less than 2% variation in total soil weight. This resulted in a bulk density of approximately 1.35 g cm^{-3} across all cylinders.

The cylinders were saturated, weighing 163–165 kg when at field capacity. The soil was fertilized with a mix of diammonium phosphate, muriate of potash (200 mg kg^{-1}), and sieved, sterilized farmyard manure at 1:25 ratio (v/v) to provide essential nutrients. The lysimeters were spaced about 10 cm apart. Sorghum millet was planted using a field-typical spacing of 60 cm × 10 cm to mirror natural growth. The lysimeters were set up in six deep, wide trenches, giving the plants a similar soil volume as in the field (Fig. 2). This lysimeter approach enabled detailed monitoring of plant water use and biomass accumulation from early growth to maturity, allowing for highly accurate transpiration efficiency assessments with minimal error, making it ideal for long-term studies. Tracking transpiration throughout the full crop cycle avoided the issues of short-term experiments where transpiration can vary significantly.

Screening for shoot fly resistance by interlard-fish meal technique

In addition to evaluating the water use efficiency traits, the same set of 45 genotypes were also assessed for their resistance to shoot fly. The screening was conducted across four different hotspot locations (Parbhani, Solapur, Vijayapura, Dharwad) in the states of Maharashtra and Karnataka during the post-rainy season of 2023. To ensure uniform shoot fly pressure under field conditions, the interlard-fish meal technique[47] was employed. A susceptible cultivar, Swarna, was sown 20 days prior to the test material to allow for shoot fly population build-up. Seven days after seedling emergence, moistened fish meal was spread uniformly in four blocks covering the test genotypes to attract the emerging shoot flies from the infester rows. Plant protection measures were avoided

Fig. 2. A lysimeter facility with a rain-out shelter at the ICAR-Indian Institute of Millets Research, Hyderabad to screen the genotypes and evaluate their performance under drought conditions. The lysimeter method (long PVC tubes of 2.0 m length and 25 cm diameter) enabled to closely monitor and record the plant water use patterns and biomass accumulation of the tested genotypes from the early growth stages all the way through to maturity. This detailed data collection and analysis approach provided valuable insights into the drought response mechanisms and adaptive strategies of the different genotypes under investigation.

until the shoot fly infestation period was complete. The following parameters were recorded to assess shoot fly incidence:

Shoot fly (% dead hearts)
The incidence of dead hearts, which indicates shoot fly infestation, was assessed at 21 days after seedling emergence (DAE). The dead heart occurrence was quantified as a percentage, calculated by dividing the number of shoots exhibiting dead hearts by the total number of shoots, and then multiplying by 100. This provided a measure of the severity of shoot fly attack on the test genotypes. The genotypes were then classified into different resistance categories based on the following established rating scale developed by Nimbalkar and Bapat in 1987[48].

Rating	Reaction	
0–10% dead heart	Highly resistant	HR
10–20% dead heart	Resistant	R
20–30% dead heart	Moderately resistant	MR
30–50% dead heart	Susceptible	S
Above 50% dead heart	Highly susceptible	HS

Glossiness
Leaf glossiness was visually scored on a 1 to 5 scale at 7 DAE, where a score of 5 indicated leaves with a highly glossy, reflective surface, and a score of 1 denoted non-glossy, dull leaves. This leaf glossiness trait was assessed during the morning hours when the leaves exhibited maximum light reflection[49].

Seedling vigour
The seedling vigor of the test genotypes was assessed and scored at 16 16 DAE. The scoring was done on a 1 to 5 scale, where a score of 5 indicated plants with high vigor, exhibiting robust growth and development, and a score of 1 denoted plants with low vigor, displaying poor seedling establishment[49].

Screening for charcoal rot resistance by sick-plot method

The 2-way, 4-way and 8-way cross derived lines, along with their founder lines, were evaluated for charcoal rot resistance in three hotspot locations (Parbhani, Solapur and Dharwad) across the states of Maharashtra and Karnataka during the post-rainy season of 2023. The test lines were grown in a replicated field trial established within a Macrophomina-infested 'sick-plot' (the soil had an inoculum density of 100 to 150 microsclerotia per gram). A susceptible (CSV 8R) and resistant checks (E 36-1) of similar maturity were included to compare disease reaction (repeated after every 20 lines). Uniform plant spacing was maintained, and uniform soil moisture stress was induced after flowering by withholding irrigation and/or removing the flag leaf. The number of charcoal rot-infected plants, lesion length, number of nodes crossed by lesion, and grain yield at harvest were also recorded. Charcoal rot incidence was measured as the percentage of plants exhibiting charcoal rot symptoms in each entry [CRP = (Number of charcoal rot infected plants/Total number of plants) × 100]. Severity was assessed by measuring the mean length of lesion spread (MLS, cm) in the stalks. Since both incidence and severity are crucial in determining a line's charcoal rot resistance or susceptibility, a charcoal rot index

(CRI) was calculated by combining these two factors using a formula (CRI = CRP × 0.4 + MLS × 0.6). The disease reaction was then categorized based on the CRI values[50]. ranging from Highly resistant – HR (< 5) to Highly Susceptible-HS (> 40), with intermediate categories of Resistant-R (5–10), Moderately Resistant-MR (11–25), and Susceptible-S (26–40).

Stay green is an important character that imparts charcoal rot resistance to sorghum genotype. Top five leaves were observed for greenness at the time of physiological maturity and a visual stay green rating of 1–5 was followed to categorize the genotypes.

Rating	Description
1	Indicates completely green normal sized leaves (no leaf death)
2	25% of the leaves died
3	26–50% of the leaves died
4	51–75% were dead
5	76–100% of the leaves and stalk were dead (complete plant death)

Data analysis

The two-season field data on the eleven measured phenotypic traits was statistically analyzed using the analysis of variance (ANOVA) for the alpha lattice experimental design, as developed by Patterson and Williams (1976)[51]. Additionally, the R software package grapesAgri1 (version 0.1.0), developed by Gopinath et al. in 2021[52], was utilized to perform a comprehensive statistical analysis of the collected data.

The linear model of observations in alpha design is as follows:

$$\mathrm{yijk} = \mu + \mathrm{t_i} + \mathrm{r_j} + \mathrm{b_{jk}} + \mathrm{e_{ijk}}.$$

where, y_{ijk} denotes the value of the observed trait for ith treatment received in the kth block within jth replicate (superblock), t_i is the fixed effect of the ith treatment (i = 1,2,…,t); r_j is the effect of the jth replicate (superblock) (j = 1,2,…,r); b_{jk} is the effect of the kth incomplete block within the jth replicate (k = 1,2,…s) and e_{ijk} is an experimental error associated with the observation of the ith treatment in the kth incomplete block within the jth complete replicate.

The lysimeter data on drought tolerant traits were analyzed using the OPSTAT (http://14.139.232.166/opstat/default.asp) software program. The four-location data on shoot fly incidence and the three-location data on charcoal rot incidence were subjected to homogeneity testing using the Bartlett test to ensure the reliability of the data, as the data did show heterogeneity of variance across the test locations. This homogeneity testing was conducted to verify that the data collected from the multiple locations could be pooled and analyzed together to provide a comprehensive assessment of the genotypes' performance for these two important traits.

The trait means, coefficient of variation, standard error, and other relevant statistical parameters were calculated using the Traitstats (version 0.1.0) R software package developed by Nitesh et al. in 2020[53]. This software provided a robust statistical analysis of the data, generating key metrics that enabled a comprehensive evaluation of the genotypes' performance. Additionally, the data was visually represented through bar charts and graphs, which were prepared using Microsoft Excel. These visualizations complemented the statistical analysis, providing a clear and accessible means to interpret the results in detail and draw meaningful conclusions from the study.

Results

Performance of multi-parent cross derivatives (MPCDs) under field trials

ANOVA and mean performance

The analysis of variance of the 11 phenotypic traits evaluated over the 2-year field trials revealed significant differences among the test entries for most characteristics, except for panicle exertion and test weight. The mean values, standard error, minimum, maximum, and coefficient of variation for the 2-way, 4-way and 8-way cross derived lines (MPCDs) and, 17 founder parents of all the studied traits are presented in Table 3. The findings demonstrated high variability in the measured traits, with the highest coefficient of variation observed for grain yield (CV = 41%), followed by panicle yield (CV = 36%) and peduncle length (CV = 20%). The means of grain yield varied considerably, ranging from 1014.15 to 5983.54 kg/ha. Similarly, stover yield ranged from 5119.34 to 11,728.40 kg/ha, and test weight varied from 2.75 to 4.24 g per 100 seeds. This substantial range of variation observed for these dual-purpose characteristics in post-rainy season sorghum presents an excellent opportunity for further improving yield and enhancing the crop's overall productivity.

Among the 28 test entries, the lines originated from the 8-way crosses demonstrated the highest significant advantages in terms of grain and stover production compared to their founder lines and check entries. Specifically, the 8-way cross-3 (S22086RV) exhibited the highest grain yield (5983.54 kg/ha), stover yield (11,728.40 kg/ha), and 100-seed weight (4.24 g), followed by the 8-way cross-2 (S22085RV) with a grain yield of 5580.25 kg/ha, and the 8-way cross-4 (S22087RV) with a grain yield of 5465.02 kg/ha and a stover yield of 11,028.81 kg/ha (Table 3). These 8-way cross-derived lines also displayed favorable agronomic traits, including early maturity, medium height, and long panicles, in comparison to the 2-way and 4-way cross derivatives as well as their parental lines. Furthermore, the 4-way cross derivatives exhibited superior performance compared to the 2-way cross derivatives, which were developed from only two parents.

Identities	DFF	DM	PH	PE	Ped. L	PL	PW	PWt	GY	SY	TW
S22060RV	73	123	218	13.25	19.20	10.50	3.22	5308.64	3580.25	8485.60	2.97
S22061RV	66	117	238	9.30	25.95	13.67	2.72	4938.27	3950.62	7983.54	2.89
S22062RV	76	126	234	15.08#	20.55	17.50	4.02#	4691.36	2962.96	8189.30	2.87
S22063RV	76	127	252	11.45	16.25	13.67	2.78	4938.27	4135.80	8107.00	2.84
S22064RV	73	124	234	14.20	20.05	11.00	3.62	5555.56	3641.98	8596.71	2.94
S22065RV	78	128	241	9.00	26.05	18.17#	3.47	5802.47	5135.80#	9020.58	2.92
S22066RV	74	126	229	13.88	27.15	15.50	2.67	5432.10	3604.94	8530.04	2.97
S22067RV	68	118	275	11.55	17.65	14.67	2.83	5061.73	4493.83	8452.68	3.05
S22068RV	69	121	254	13.85	24.25	13.50	3.67	5308.64	3629.63	8905.35	3.00
S22069RV	75	126	238	11.05	20.45	11.17	2.80	4938.27	4135.80	7860.08	2.88
S22070RV	75	126	242	9.98	29.63#	17.00	2.43	5061.73	3234.57	8060.91	2.99
S22071RV	72	123	260	8.18	28.90#	14.17	3.52	5555.56	5197.53#	8798.35	2.82
S22072RV	78	126	237	17.00#	28.90#	16.00	3.27	5308.64	3456.79	8460.91	2.82
S22073RV	76	126	274	12.20	18.10	16.67	2.87	5802.47	5209.88#	9069.96	2.82
S22074RV	76	126	262	15.13	25.97	15.00	3.53	4814.82	4148.15	7878.19	2.98
S22075RV	77	125	276	10.27	23.43	17.17	2.27	5061.73	4320.99	7983.54	2.98
S22076RV	69	119	231	10.07	18.50	10.00	3.13	5061.73	3333.33	8074.08	2.84
S22077RV	75	124	231	13.75	26.90	13.67	2.95	6049.38	4098.77	9432.10#	2.84
S22078RV	74	125	222	15.17#	21.95	10.50	2.98	5555.56	4074.07	8407.41	2.76
S22079RV	68	116	250	14.60	26.13	15.50	3.12	5679.01	4753.09	8641.98	2.72
S22080RV	72	122	261	11.17	29.72#	16.00	3.53	5308.64	3518.52	8308.64	3.01
S22081RV	68	118	244	11.87	24.93	16.67	3.33	5802.47	4629.63	9012.35	3.18
S22082RV	75	124	241	14.88	23.90	14.67	2.95	4938.27	3543.21	7604.94	2.80
S22083RV	76	125	240	14.00	20.75	13.50	2.58	5873.55	4827.16	9116.35	2.93
S22084RV	68	118	234	9.88	25.87	17.50	3.27	5950.62	4617.29	8888.89	3.19
S22085RV	67#	117	230	15.02#	27.37	15.50	3.57	7888.89#	5580.25#	10,271.61#	3.36#
S22086RV	67#	112	218	16.18#	32.40#	19.20#	4.48#	8436.21#	5983.54#	11,728.40#	4.24#
S22087RV	73	123	257	14.97	29.65#	20.50#	3.53	6944.44	5465.02#	11,028.81#	3.35#
CSV 14R	74	120	196	12.62	16.67	15.38	3.08	4304.86	2692.18	7345.68	2.97
Solapur Dagadi	75	121	193	14.17	17.33	15.03	4.02	2904.53	1174.08	6823.05	3.04
CRS 4	68	122	150	13.45	18.17	17.38	3.17	1370.86	1041.15	6831.28	3.09
Phule Revati	73	120	234	11.05	17.00	19.23	4.30	3057.20	1348.15	7613.17	3.04
CSV 22	73	121	204	13.55	17.33	18.07	3.88	4378.60	2744.86	8493.83	2.90
Phule Chitra	71	121	236	12.25	18.17	17.95	3.32	2629.63	1930.45	6487.24	3.28
PKV Kranti	70	119	243	12.37	33.00	18.00	4.73	1581.90	1199.18	5257.20	3.42
Phule Suchitra	70	121	208	10.90	18.67	16.47	3.62	2000.00	1530.87	5119.34	2.97
CSV 216R	69	120	314	11.25	22.00	20.27	3.50	1736.63	1222.22	5255.15	2.83
Parbhani Moti	75	122	224	11.18	21.33	17.98	3.67	3103.70	1985.19	7148.56	3.07
CRS 20	76	119	238	10.75	22.00	18.62	4.82	1874.49	1578.60	6397.53	3.62
DSV 5	75	123	251	9.67	25.33	16.88	3.23	2300.41	1810.70	7016.46	3.14
Selection 3	64	114	172	13.63	18.97	19.29	3.02	2413.58	1217.29	6983.54	2.90
DSV 4	74	119	228	13.15	25.67	18.87	4.15	2817.28	1572.02	6802.47	3.27
CSV 29R (NC)	75	124	246	13.45	27.20	18.83	2.65	5230.45	3563.79	8884.77	3.43
CSV 26R (NC)	73	123	235	12.83	27.17	15.80	4.63	3860.98	2621.40	7181.07	3.26
M35-1 (PLC)	74	124	254	10.70	28.87	16.11	3.55	3465.02	2670.78	7189.30	3.48
Continued											

Identities	DFF	DM	PH	PE	Ped. L	PL	PW	PWt	GY	SY	TW
Mean	72.51	121.87	236.64	12.53	23.45	15.97	3.39	4579.98	3359.25	8038.40	3.06
SE	0.53	0.54	4.05	0.31	0.69	0.39	0.09	243.90	207.67	197.45	0.04
Min	64.00	112.00	150.00	8.18	16.25	10.00	2.27	1370.86	1014.15	5119.34	2.72
Max	78.00	128.00	314.00	17.00	33.00	20.50	4.82	8436.21	5983.54	11,728.40	4.24
CV (%)	5.0	3.0	11.0	17.0	20.0	17.0	18.0	36.0	41.0	16.0	9.0

Table 3. This table presents the performance of lines derived from 2-way, 4-way, and 8-way crosses, along with their founder lines, for key phenotypic traits evaluated across two consecutive post-rainy seasons in 2022 and 2023. The phenotypic traits assessed include: DFF: Days to 50% flowering; DM: Days to maturity; PH: Plant height in cm; PE: Panicle Exertion in cm; Ped.L: Peduncle Length in cm; PL: Panicle length in cm; PW: Panicle width in cm; PWt: Panicle weight in kg/ha; GY: Grain yield in kg/ha; SY: Stover yield in kg/ha; TW: 100 seed weight in gm. NC stands for National Check, and PLC stands for Popular Local Check. The top performing entries for the respective traits are highlighted with the # symbol, indicating that these entries have demonstrated the highest or most desirable values for the specific phenotypic characteristics evaluated in the study. Descriptive statistical parameters are presented as SE: Standard error; Min: Minimum; Max: Maximum; CV: Coefficient of variation.

Percent superiority of MPCDs over 2-way, 4-way cross derivatives and their founders

The average mean performances of the sixteen 2-way, eight 4-way, and four 8-way cross derived lines, along with their 17 founder lines, were estimated separately and compared to assess their superiority in terms of grain and stover yields (Fig. 3a) (Table S1). The results demonstrated that the MPCDs, exhibited the highest overall average performance, surpassing the 4-way and 2-way cross derived lines, as well as the founder parental lines, for both grain and stover yields. Notably, the MPCDs showed the highest percent superiority, with 188.36% and 52.49% for grain and stover yields, respectively, over their founder lines (Fig. 3b). Furthermore, they exhibited over 70% and 30% higher grain and stover yields, respectively, compared to their bi-parent (2-way) cross derived lines. The average mean performances of the 4-way cross derivatives were found to be on par with those of the 2-way cross derivatives. However, the 8-way cross derivatives exhibited significantly higher grain and stover yields in comparison to the 2-way cross derivatives, indicating their enhanced potential for improved productivity.

Response of MPCDs for drought and water use efficiency (WUE) traits

The lysimeter experiment revealed significant differences among the test entries in various water use parameters, such as transpiration rate (TR), transpiration efficiency (TE), and total water use (TWU). It also showed differences in yield-related components, including shoot biomass, harvest index, and grain yield, under both well-watered and water-stressed conditions. The 8-way cross S22086RV exhibited the highest TR and TE, followed by S22085RV, S22087RV, and S22084RV. Meanwhile, S22086RV displayed the highest shoot biomass and harvest index, while S22087RV achieved the highest grain yield under both moisture regimes (Table 4).

The 8-way cross derivatives demonstrated lower total water use compared to the other test entries, suggesting their enhanced water use efficiency. The transpiration rate exhibited the highest variability under both well-watered (range = 65.75) and water-stressed (range = 75.6) conditions among the water use traits. Similarly, shoot biomass showed the highest variability for the yield component traits under both moisture regimes, followed by grain yield. The 8-way cross-derived lines outperformed the 2-way, 4-way, and parental entries. Genotypes (S22086RV, S22085RV) with high TR, TE, and TWU efficiency were strongly associated with better shoot biomass accumulation, high grain yield, and harvest index. The lines that performed well under both well-watered and water-stressed conditions are considered responsive and could benefit from increased irrigation in post-rainy sorghum cultivation.

Reaction of MPCDs against shoot fly incidence

Reaction of the MPCDs along with their 2-way, 4-way crosses and their founder lines, as well as resistant checks, revealed that the 8-way cross-derived lines exhibited lower incidences of shoot fly infestation compared to the corresponding 4-way and 2-way cross derivatives, as well as the founder lines (Table 5). This suggests that the 8-way cross derivatives may possess enhanced levels of resistance against the shoot fly pest. The entries S22086RV and S22087RV demonstrated a resistant reaction with 11.72% and 12.43% dead hearts, respectively, indicating their potential as valuable sources of shoot fly resistance that could be leveraged in future breeding efforts. In contrast, the susceptible check Swarna showed a highly susceptible reaction with 53.51% dead hearts, while the resistance check IS 18,551 exhibited 14.28% dead hearts, suggesting sufficient infestation of the pest at the target locations. Interestingly, the other two 8-way cross derivatives and a few of the 4-way cross derivatives also displayed acceptable levels of shoot fly resistance, further highlighting the superiority of the lines having more than two parents in combination, compared to the bi-parent cross derivatives and the founder parents. The genotypes with shoot fly dead heart percentage anything less than 25%, a benchmark set in post-rainy sorghum, are considered valuable and could be used in future breeding programs to improve shoot fly resistance in sorghum.

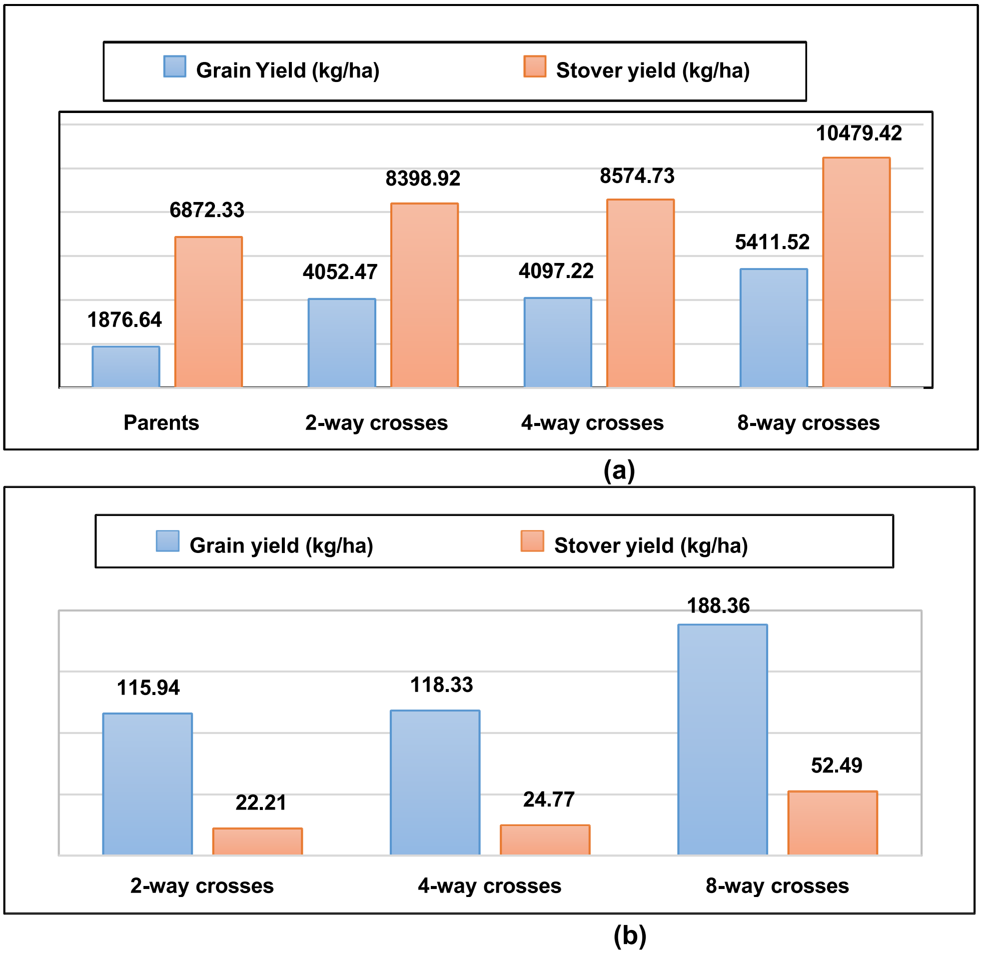


Fig. 3. Provides a comparison of the performance of 8-way cross derived lines with their 4-way and 2-way cross derivatives, as well as the founder parent lines, in terms of both grain yield and stover yield. (**a**) The average performance data is presented for a total of 17 parent lines, sixteen 2-way, eight 4-way and four 8-way cross derived lines. Additionally, this figure highlights the (**b**) average percent superiority of the 2-way, 4-way, and 8-way cross derived lines compared to the founder lines.

Reaction of MPCDs against charcoal rot disease

The reaction of the 2-way, 4-way, and 8-way cross-derived lines, along with their founder lines and a resistant check, were evaluated across three hot spot locations to assess their response to charcoal rot and associated traits. The results are presented in Table 6. The charcoal rot index (CRI) values ranged from 8.9 to 23.0 among the test entries, including the resistant check E-36-1. Interestingly, the two MPCDs (8-way cross derivatives), S22086RV (CRI = 8.9) and S22085RV (CRI = 9.8), exhibited a resistant reaction, while the rest of the entries displayed a moderately resistant response. Furthermore, the 8-way cross derivatives demonstrated superior performance in terms of the stay-green trait and lodging percentage when compared to their corresponding 2-way and 4-way cross derivatives, as well as the parental lines. Notably, the entry S22086RV exhibited the best stay-green trait expression along with the lowest lodging percentage, indicating its potential advantage for charcoal rot resistance over the other entries. Similarly, the other three 8-way cross-derived lines also showed better values for stay-green and lodging, further highlighting the enhanced performance of these multi-parent cross derivatives.

Discussion

Potential of multi-parent crossing in developing dual purpose sorghum genotypes

The dual-purpose (grain and fodder) nature and drought tolerance of post-rainy sorghum have established it as an economically viable and suitable crop for the post-rainy season, with limited alternatives. Additionally, post-rainy sorghum plays a pivotal role in ensuring household and regional food and nutritional security, particularly in arid and semi-arid regions where it is a staple crop[54]. The regional significance of post-rainy sorghum as a food crop is comparable to that of wheat and rice at the national level. However, genetic gains in post-rainy sorghum have been constrained by a lack of phenotypic variability and limited response to selection, hindering improvements in crop productivity. The availability of a broader genetic base for any given trait is crucial for a successful trait improvement program[54]. Therefore, breeding efforts focused on developing farmers' preferred varieties possessing dual-purpose capabilities, bold seeds, medium maturity, resistance to lodging, drought

Identity	Transpiration rate (g/day/plant)		Transpiration efficiency (g/day/plant)		Total water use (kg/plant)		Shoot biomass (g/plant)		Harvest index (g/plant)		Grain yield (g/plant)	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
S22060RV	101.20	97.47	6.21	7.89	22.65	18.79	94.69	78.87	0.40	0.37	61.38	41.14
S22061RV	95.08	68.78	6.19	7.46	22.70	18.80	100.87	88.36	0.38	0.33	62.05	43.14
S22062RV	96.37	69.97	4.42	6.98	23.03	20.15	101.36	88.77	0.37	0.30	63.45	43.12
S22063RV	85.23	67.50	6.25	8.20	20.89	17.54	102.06	89.24	0.41	0.39	52.32	36.58
S22064RV	100.99	80.35	6.33	8.40	20.56	16.56	125.40	98.81	0.38	0.33	54.87	37.04
S22065RV	101.60	84.72	6.50	8.45	19.87	15.65	126.89	99.65	0.38	0.33	71.80	48.57
S22066RV	102.61	85.33	6.69	8.54	20.11	15.68	127.10	100.45	0.39	0.33	72.06	48.77
S22067RV	104.55	86.77	6.75	8.68	19.57	15.14	136.30	100.86	0.39	0.34	74.47	50.04
S22068RV	109.40	86.70	6.85	8.74	18.56	12.44	139.57	102.58	0.41	0.36	75.26	50.14
S22069RV	110.20	89.85	7.05	9.10	18.65	12.65	147.49	106.22	0.45	0.37	76.14	50.94
S22070RV	111.25	90.34	7.20	9.25	19.44	12.68	152.32	106.94	0.22	0.15	63.47	44.45
S22071RV	114.36	94.60	7.22	9.30	19.45	13.50	154.87	112.02	0.28	0.22	65.25	46.58
S22072RV	100.56	79.95	5.77	7.21	20.60	16.57	104.80	90.04	0.32	0.24	66.22	47.75
S22073RV	99.20	75.35	5.79	7.40	20.71	16.82	105.65	90.50	0.34	0.27	69.10	48.36
S22074RV	100.30	78.90	5.35	7.16	20.80	17.44	107.02	91.34	0.35	0.27	55.65	37.13
S22075RV	99.77	76.98	6.00	7.42	21.56	17.56	115.47	93.12	0.36	0.28	58.57	39.45
S22076RV	115.89	95.30	7.40	9.33	18.50	11.77	158.57	116.13	0.52#	0.48#	78.89	59.65
S22077RV	117.24	95.78	7.49	9.37	18.54	12.24	161.38	130.04	0.51#	0.41	78.82	58.36
S22078RV	121.47#	99.97	7.80	9.60#	16.04	11.35	169.10	137.13	0.53#	0.41	79.58	60.81
S22079RV	123.21#	100.50#	7.61	9.60#	16.21	11.45	172.58#	139.45	0.53#	0.44	82.34#	61.75#
S22080RV	124.57#	102.15#	8.04#	9.84#	15.82	11.24	178.82#	150.14	0.53#	0.45#	86.30#	65.25#
S22081RV	123.57#	101.60#	7.80	9.64#	15.46	10.36	186.14#	151.57	0.54#	0.47#	87.02#	68.87#
S22082RV	120.68#	97.56	7.50	9.51#	17.24	11.47	162.05	134.57	0.55#	0.48#	87.36#	70.86#
S22083RV	118.35	96.33	7.50	9.47	18.26	11.68	163.47	136.58	0.55#	0.48#	77.10	51.57
S22084RV	124.87#	102.98#	8.45#	9.96#	14.44#	10.01#	193.54#	158.36#	0.56#	0.54#	89.65#	76.24#
S22085RV	130.54#	116.80#	8.60#	10.04#	13.65#	9.51#	196.70#	169.03#	0.58#	0.58#	90.87#	76.83#
S22086RV	133.25#	118.11#	8.65#	10.07#	11.65#	8.35#	199.65#	185.20#	0.59#	0.58#	92.70#	85.20#
S22087RV	130.11#	110.40#	8.86#	10.47#	15.37#	10.25#	195.26#	165.25#	0.57#	0.55#	94.69#	85.34#
CSV 14R	102.30	98.50	3.52	6.87	20.89	19.10	82.34	67.75	0.20	0.15	39.57	22.58
Solapur Dagadi	80.71	58.97	3.24	5.47	19.45	17.89	83.45	71.14	0.29	0.27	47.49	28.13
CRS 4	76.98	54.62	3.54	6.87	21.47	18.78	80.24	75.68	0.30	0.28	43.51	27.25
Phule Revati	80.35	55.98	4.50	7.51	20.01	19.45	79.57	78.97	0.31	0.26	38.72	34.22
CSV 22	98.57	62.57	3.22	5.10	24.66	20.38	85.78	69.87	0.22	0.16	45.22	41.25
Phule Chitra	78.24	45.88	6.89	8.01	25.57	20.65	71.20	65.48	0.22	0.15	50.87	33.89
PKV Kranti	87.45	70.50	4.75	6.78	25.01	22.47	80.11	76.48	0.28	0.22	49.78	41.70
Phule Suchitra	98.00	68.54	4.87	7.89	20.20	18.46	77.48	67.89	0.27	0.20	50.10	40.57
CSV 216R	67.50	50.40	3.45	7.51	19.87	18.50	86.32	74.56	0.25	0.21	45.78	37.10
Parbhani Moti	77.54	57.43	4.78	6.45	23.10	21.10	81.57	78.98	0.28	0.24	42.98	33.28
CRS 20	69.80	42.51	3.10	5.79	20.47	19.02	85.77	80.18	0.29	0.26	38.01	27.80
DSV 5	100.23	93.56	5.98	8.04	22.78	20.17	80.57	74.56	0.30	0.25	43.78	26.56
Selection 3	99.40	78.54	6.87	8.00	23.57	21.22	72.34	69.78	0.32	0.27	51.46	48.75
DSV 4	87.45	56.87	4.20	7.55	24.19	22.28	76.58	70.46	0.21	0.19	50.98	30.54
CSV 29R (NC)	108.22	88.90	6.98	8.44	19.18	18.56	70.89	68.42	0.34	0.30	51.12	33.56
CSV 26R (NC)	99.87	73.25	5.79	7.46	21.87	19.67	82.42	73.21	0.29	0.24	44.10	28.78
M35-1 (PLC)	87.50	64.87	6.78	8.27	20.06	19.88	73.64	62.54	0.33	0.28	49.00	29.35

Continued

Identity	Transpiration rate (g/day/plant)		Transpiration efficiency (g/day/plant)		Total water use (kg/plant)		Shoot biomass (g/plant)		Harvest index (g/plant)		Grain yield (g/plant)	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
Mean	102.59	81.62	6.19	8.20	19.84	16.12	120.65	100.83	0.38	0.33	63.33	46.65
SE	2.50	2.83	0.24	0.19	0.46	0.60	6.26	4.84	0.017	0.018	2.52	2.38
Min	67.50	42.51	3.10	5.10	11.65	8.35	70.89	62.54	0.20	0.15	38.01	22.58
Max	133.25	118.11	8.86	10.47	25.57	22.47	199.65	185.20	0.59	0.58	94.69	85.34
CV (%)	16.0	23.0	26.0	16.0	15.0	25.0	35.0	32.0	31.0	36.0	27.0	34.0

Table 4. Performance of 2-way, 4-way and 8-way cross derived lines for key water use traits contributing to drought stress tolerance. Traits such as Transpiration Efficiency, Transpiration Rate and Total Water Use were the estimated parameters. Shoot biomass and grain yield were the recorded traits measured. WW stands for Well-watered; WS for Water stressed. The top performing entries for the respective traits are highlighted with the # symbol. The descriptive statistical parameters calculated include Mean, Standard Error, Minimum, Maximum and Coefficient of Variation.

tolerance, and resistance to pests like shoot fly and diseases like charcoal rot become essential to achieve the much-needed area expansion and profitability in post-rainy sorghum.

While modern agriculture, based on biparental crop varieties, has significantly contributed to the world's food supply, this strategy is also facing challenges due to stagnation in yield growth, the increasing impact of climate change, and susceptibility to biotic and abiotic stresses[18]. To address these challenges and further enhance the productivity and resilience of post-rainy sorghum, it is crucial to expand the genetic base through the use of multi-parent crosses that can combine favorable traits from diverse parents, leading to enhanced drought tolerance, increased yields, and better adaptability to changing environmental conditions. While bi-parent crossing involving only two parents may be simpler to implement, it is inherently limited in its ability to fully harness the genetic diversity available within a crop species due to the restricted number of parental lines involved. In contrast, multi-parental crossing integrates the genetic contributions of more than two parents, leading to a broader genetic diversity and potentially enhanced traits in the offspring[56]. Crossing more than two parental lines, such as in 4-way and 8-way crosses, offers several advantages over traditional bi-parental crossing approaches[57]. Multi-parent crosses can capture a broader range of genetic variation, potentially leading to more robust and adaptable plant varieties.

In the current study, we have successfully demonstrated the immense potential of the multi-parent crossing strategy in post-rainy sorghum for developing genotypes with enhanced yield levels, as well as improved resilience against key biotic and abiotic stresses such as drought, shoot fly, and charcoal rot. The developed MPCD (8-way cross) lines exhibited superior agronomic performance compared to that of the 4-way and 2-way cross-derived lines, as well as the original founder genotypes. This superior performance can be directly attributed to the increased genetic diversity present in the 8-way crosses, which provides a broader pool of allelic variation and the potential for favorable epistatic interactions[41]. The elevated recombination events in the 8-way crosses can lead to the expression of beneficial allelic combinations and the uncovering of transgressive segregation, ultimately resulting in the observed superior agronomic performance[58]. Othe possible explanation for the enhanced performance of these lines is the increased opportunity to capture favorable gene combinations and break down undesirable linkages that can occur with a larger number of founder lines[34].

The wide range of variation observed in the key agronomic traits, such as grain and stover yields, test weight, and panicle weight, among the evaluated genotypes suggests the immense potential to identify superior performing lines through the multi-parent crossing approach employed in this study. This potential is further evidenced by the outstanding mean performances of two distinct 8-way cross derivatives, namely S22086RV and S22085RV, which have significantly surpassed the 2-way and 4-way cross derivatives, as well as the founder genotypes, in terms of both grain and stover yields (Fig. 4a,b) (Table S2). The enhanced levels of grain and stover in these genotypes were likely the result of the expression of individual yield-governing traits. The contrasting parents, with differences in trait expression, coming together and recombining more often led to a cumulative increase in the trait values. This combination and recombination of diverse genetic backgrounds from the contrasting parental lines contributed to the observed enhanced grain and stover yields in the multi-parent cross-derived lines. Aggregating several of the best performing lines, each possessing favorable trait expression, can potentially result in the creation of outstanding progenies with enhanced productivity[59].

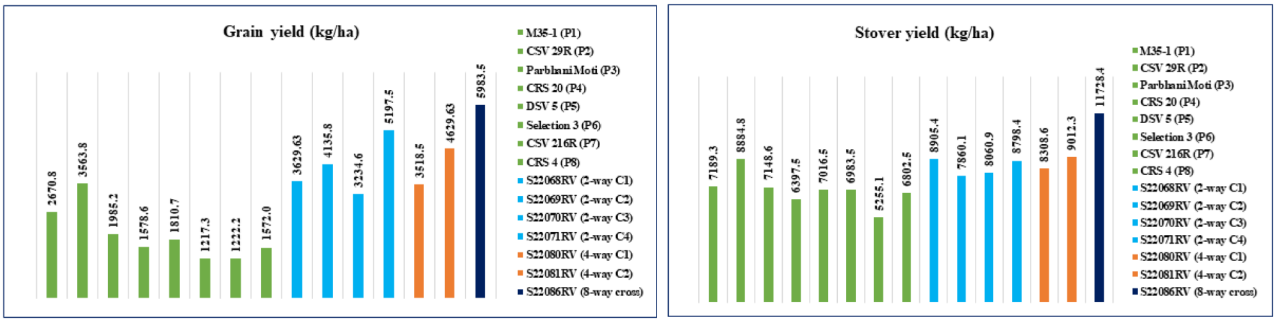
Notably, the 8-way cross-derived lines demonstrated over 70% and 30% grain and stover yield advantages, respectively, compared to the bi-parent crosses. Specifically, two exceptional 8-way cross-derived lines, S22086RV and S22085RV, outclassed the national check cultivar CSV 29R by nearly 70% and 60% in grain yield, and over 30% and 15% in stover yield, respectively. Furthermore, the 8-way cross derivatives also exhibited favorable agronomic traits, including early maturity, medium plant height, and long panicles, in comparison to the 2-way and 4-way cross derivatives. These results clearly highlight the immense potential of the multi-parent crossing strategy for developing high-performing post-rainy sorghum cultivars that can significantly boost grain and biomass production, contributing to enhanced food security and profitability for farmers in the target regions[60,61]. The superior performance of the 8-way cross derivatives, with their combination of improved yield, stress tolerance, and desirable agronomic characteristics, underscores the value of this breeding approach

Type of cross		Identity	Shoot fly (% DH)	Seedling glossiness	Seedling vigor	Reaction
2-way cross derivatives	1	S22060RV	28.03	2.9	2.3	MR
	2	S22061RV	27.10	2.8	2.1	MR
	3	S22062RV	23.80	2.8	2.1	MR
	4	S22063RV	24.90	3.2	2.2	MR
	5	S22064RV	21.70	2.8	2.2	MR
	6	S22065RV	23.50	2.8	2.2	MR
	7	S22066RV	24.30	2.9	2.3	MR
	8	S22067RV	23.90	3.0	2.5	MR
	9	S22068RV	23.30	3.0	2.5	MR
	10	S22069RV	23.60	3.0	2.2	MR
	11	S22070RV	21.20	2.8	2.3	MR
	12	S22071RV	21.60	2.9	2.5	MR
	13	S22072RV	26.40	2.7	2.2	MR
	14	S22073RV	25.80	2.3	2.4	MR
	15	S22074RV	26.90	2.9	2.2	MR
	16	S22075RV	26.40	2.6	2.5	MR
4-way cross derivatives	1	S22076RV	20.00	2.4	2.2	R
	2	S22077RV	20.20	2.6	2.3	MR
	3	S22078RV	19.87	2.8	2.1	R
	4	S22079RV	20.07	2.8	2.4	MR
	5	S22080RV	18.00	2.9	2.2	R
	6	S22081RV	18.10	2.7	2.5	R
	7	S22082RV	18.50	3.0	2.4	R
	8	S22083RV	19.30	2.9	2.2	R
8-way cross derivatives	1	S22084RV	18.40	2.2	2.5	R
	2	S22085RV	17.25	2.7	2.1	R
	3	S22086RV	11.72	2.0	1.9	R
	4	S22087RV	12.43	2.8	2.4	R
Parents/founder lines	1	CSV 14R	35.00	2.9	2.3	S
	2	Solapur Dagadi	31.90	2.7	2.2	S
	3	CRS 4	32.40	2.9	2.4	S
	4	Phule Revati	30.90	2.6	2.6	S
	5	CSV 22	37.50	2.6	2.6	S
	6	Phule Chitra	36.00	3.2	2.5	S
	7	PKV Kranti	29.30	3.0	2.6	MR
	8	Phule Suchitra	28.30	3.2	2.8	MR
	9	CSV 216R	29.40	3.2	3.2	MR
	10	Parbhani Moti	34.60	2.9	2.8	S
	11	CRS 20	26.20	3.0	2.8	MR
	12	DSV 5	23.90	2.8	2.8	MR
	13	Selection 3	21.80	2.7	3.1	MR
	14	DSV 4	32.90	2.8	2.6	S
	15	CSV 29R (NC)	25.20	3.1	2.6	MR
	16	CSV 26R (NC)	27.33	2.8	2.8	MR
	17	M35-1 (PLC)	25.77	3.0	2.8	MR
Resistant check		IS 18,551 (RC for SF)	14.28	2.4	1.9	R
Susceptible check		Swarna (SC for SF)	53.51	2.8	2.7	HS
Mean			25.16	2.80	2.43	–
SE			1.06	0.04	0.04	
Min			11.72	2.00	1.90	
Max			53.51	3.20	3.20	
CV (%)			29.0	9.0	12.0	–

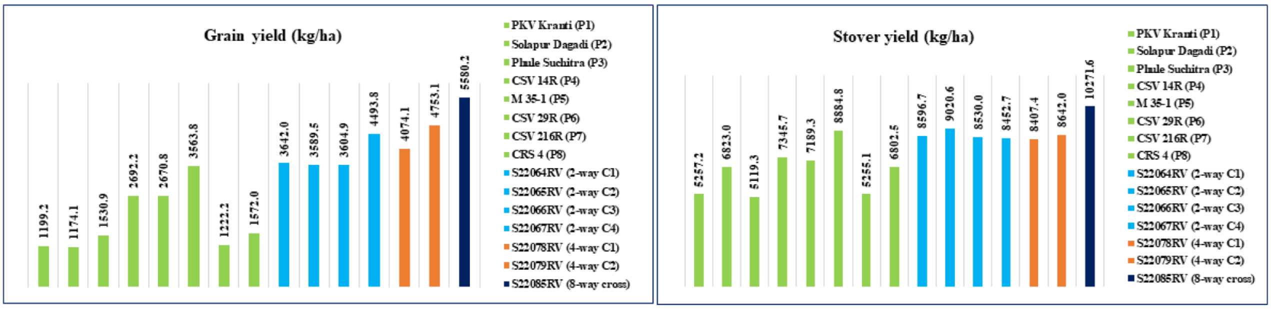
Table 5. Shoot fly incidence data for the 2-way, 4-way, and 8-way cross derived lines, evaluated alongside their parental lines and resistant check cultivars. The table includes the pooled mean values across multiple locations. The data is categorized using the following designations: DH for Dead Hearts, R for Resistant, MR for Moderately Resistant, S for Susceptible, and HS for Highly Susceptible.

Type of cross		Identity	Charcoal rot index	Stay green	Lodging (%)	Reaction
2-way cross derivatives	1	S22060RV	20.2	3	56.0 (4.1)	MR
	2	S22061RV	14.0	3	62.9 (4.8)	MR
	3	S22062RV	21.3	3	61.9 (4.7)	MR
	4	S22063RV	14.7	3	56.7 (4.2)	MR
	5	S22064RV	15.7	3	66.9 (5.1)	MR
	6	S22065RV	16.7	2	61.4 (4.6)	MR
	7	S22066RV	23.0	3	50.2 (3.9)	MR
	8	S22067RV	17.0	3	50.1 (3.8)	MR
	9	S22068RV	18.2	3	53.1 (4.1)	MR
	10	S22069RV	18.5	3	49.6 (3.8)	MR
	11	S22070RV	16.2	3	59.5 (4.7)	MR
	12	S22071RV	17.3	3	50.7 (3.9)	MR
	13	S22072RV	16.1	2	54.6 (4.1)	MR
	14	S22073RV	15.0	3	57.8 (4.3)	MR
	15	S22074RV	20.4	3	62.4 (4.8)	MR
	16	S22075RV	21.8	3	61.8 (4.7)	MR
4-way cross derivatives	1	S22076RV	21.6	3	44.5 (3.5)	MR
	2	S22077RV	18.6	2	44.0 (3.6)	MR
	3	S22078RV	16.7	3	51.5 (4.0)	MR
	4	S22079RV	16.7	3	46.8 (3.8)	MR
	5	S22080RV	16.1	2	43.2 (3.6)	MR
	6	S22081RV	17.5	2	32.4 (2.5)	MR
	7	S22082RV	15.6	3	38.2 (3.0)	MR
	8	S22083RV	15.3	3	59.0 (4.5)	MR
8-way cross derivatives	1	S22084RV	15.3	2	41.8 (3.5)	MR
	2	S22085RV	9.8	2	35.0 (2.7)	R
	3	S22086RV	8.9	2	26.5 (2.3)	R
	4	S22087RV	14.3	4	40.8 (3.3)	MR
Parents/founder lines	1	CSV 14R	18.0	3	77.5 (6.7)	MR
	2	Solapur Dagadi	17.9	3	49.0 (3.9)	MR
	3	CRS 4	14.7	3	51.1 (4.0)	MR
	4	Phule Revati	17.8	3	58.7 (4.5)	MR
	5	CSV 22	18.5	3	59.0 (4.4)	MR
	6	Phule Chitra	17.2	3	64.7 (5.0)	MR
	7	PKV Kranti	15.1	2	44.2 (3.7)	MR
	8	Phule Suchitra	16.1	3	65.0 (5.1)	MR
	9	CSV 216R	16.5	3	67.2 (5.4)	MR
	10	Parbhani Moti	16.5	3	60.8 (4.9)	MR
	11	CRS 20	20.0	4	69.7 (5.6)	MR
	12	DSV 5	15.8	3	73.8 (6.1)	MR
	13	Selection 3	17.8	4	76.8 (6.4)	MR
	14	DSV 4	16.3	4	70.2 (5.8)	MR
	15	CSV 29R (NC)	15.8	3	58.8 (4.7)	MR
	16	CSV 26R (NC)	22.3	3	46.6 (3.6)	MR
	17	M35-1 (PLC)	14.4	3	52.8 (4.0)	MR
Resistant check		E36-1	14.3	3	40.9 (3.5)	MR
Mean			16.90	2.89	54.48	–
SE			0.37	0.07	1.74	
Min			8.90	2.00	26.50	
Max			23.00	4.00	77.50	
CV (%)			17.0	18.0	21.0	–

Table 6. Charcoal rot disease incidence data for the 2-way, 4-way, and 8-way cross derived lines, evaluated alongside their parental lines and resistant check cultivars. The data includes the resistance levels for the different genetic materials, categorized as R for Resistant and MR for Moderately Resistant. Additionally, the values in the parenthesis () represent the Aitken transformed values.



a) 8-way cross-3 (S22086RV): [(M35-1 × CSV 29R) × (Parbhani Moti × CRS 20)] × [(DSV 5 × Selection 3) × (CSV 216R × CRS4)]



b) 8-way cross-2 (S22085RV): [(PKV Kranti × Solapur Dagadi) × (Phule Suchitra × CSV 14R)] × [(M 35-1 × CSV 29R) × (CSV 216R × CRS 4)]

Fig. 4. Comprehensive comparison of the mean grain and stover yields for two distinct outstanding 8-way cross derived lines (S22086RV and S22085RV), their corresponding 2-way and 4-way cross derivatives, as well as their respective founder genotypes. This figure clearly demonstrates the superior performance of the 8-way cross lines, which exhibited significantly higher grain and stover production compared to bi-parent crossing approach and the founder lines.

in unlocking the genetic potential of post-rainy sorghum and accelerating varietal improvements to meet the evolving needs of producers and consumers[62–64].

Enhanced levels of drought tolerance in MPCDs

Drought stress is one of the most significant limitations in post-rainy season sorghum production. Developing genotypes with enhanced drought tolerance is crucial to improving yield and productivity[65]. In this study, the MPCD lines demonstrated excellent water use component traits, conferring superior drought tolerance. These lines outperformed their 2-way and 4-way cross-derived counterparts, as well as the founder parents, for key water use traits such as TR, TE, TWE besides high shoot biomass, HI and grain yield. The tested lines exhibited substantial variation for all the measured traits. Notably, the MPCD lines displayed higher TE under water-stressed conditions compared to well-watered conditions, indicating a stronger drought tolerance capacity[6,66]. These lines also showed lower total water use under drought stress compared to well-watered conditions, which is a desirable trait for coping with limited water availability. The maintenance of higher biomass and harvest index under water stress, compared to well-watered conditions, for the MPCD lines suggests their ability to produce better yields even under limited water supply. The capacity to sustain higher biomass, leaf water status, and yield under drought stress is a clear indication of their drought tolerance[67]. Remarkably, nearly all the 8-way cross-derived lines, such as S22084RV, S22085RV, S22086RV, and S22087RV, as well as the 4-way cross-derived lines S22078RV, S22079RV, S22080RV, S22081RV, and S22082RV, demonstrated efficient drought-tolerant traits compared to the 2-way cross-derived lines and founder parents (Figs. 5, 6). The expression of high levels of water component traits and the observed increase in drought tolerance in the 8-way cross-derived lines can be attributed to the accumulation of favorable alleles from diverse parents and the increased genetic diversity generated through the multi-parent crossing strategy.

Multi-parent cross-derived sorghum lines that exhibit great tolerance to terminal drought stress are capable of withstanding the critical moisture deficits that often occur during the post-anthesis stage of crop development. This adaptive trait enables the plants to continue producing viable seeds and grain yields even when subjected to extreme drought conditions, thereby enhancing the overall resilience and productivity of the sorghum crop in water-limited environments. The ability of these drought-tolerant lines to sustain seed production under severe moisture stress is a valuable asset that can contribute to the stabilization of sorghum yields in regions prone to terminal drought events[68].

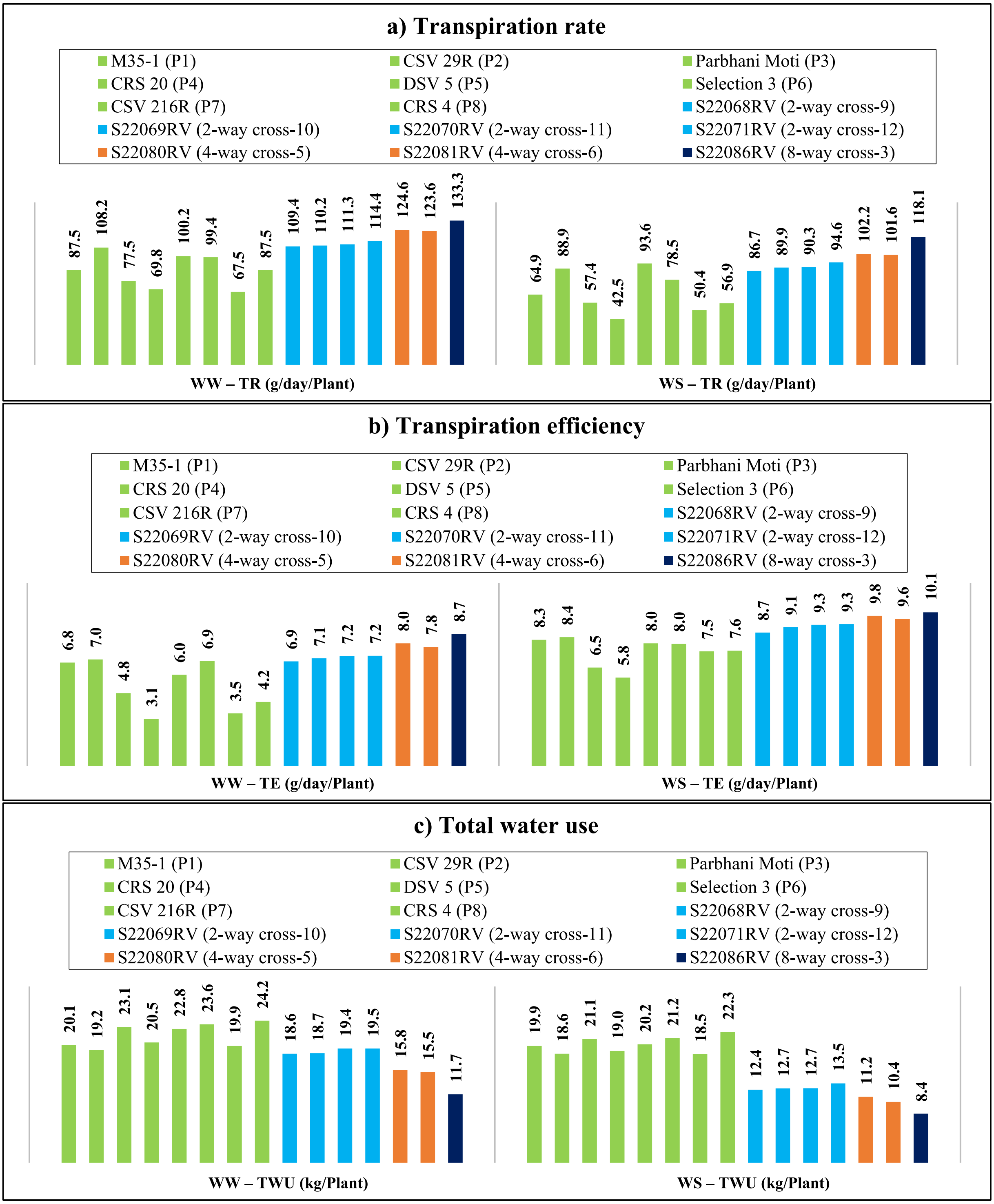


Fig. 5. Comparison of the performance of the best outstanding MPCD line, 8-way cross-3 (S22086RV), its corresponding 2-way and 4-way cross derivatives, as well as their respective founder genotypes for different water use component traits. The traits evaluated in this comparative analysis include: (**a**) transpiration rate, (**b**) transpiration efficiency, and (**c**) total water use. These parameters were assessed under both well-watered and water stress conditions using lysimetric studies, which offer valuable insights into the water use efficiency and drought tolerance characteristics of the various genetic materials tested.

MPCDs with improved shoot fly and charcoal rot resistance

Shoot fly insect-pest and charcoal rot disease are the two main yield limiting factor in post-rainy sorghum. The lines with enhanced resistance to the shoot fly and charcoal rot disease, are of great importance in sorghum improvement programs[10,11]. In this study, we thoroughly studied the levels of resistance to these two major

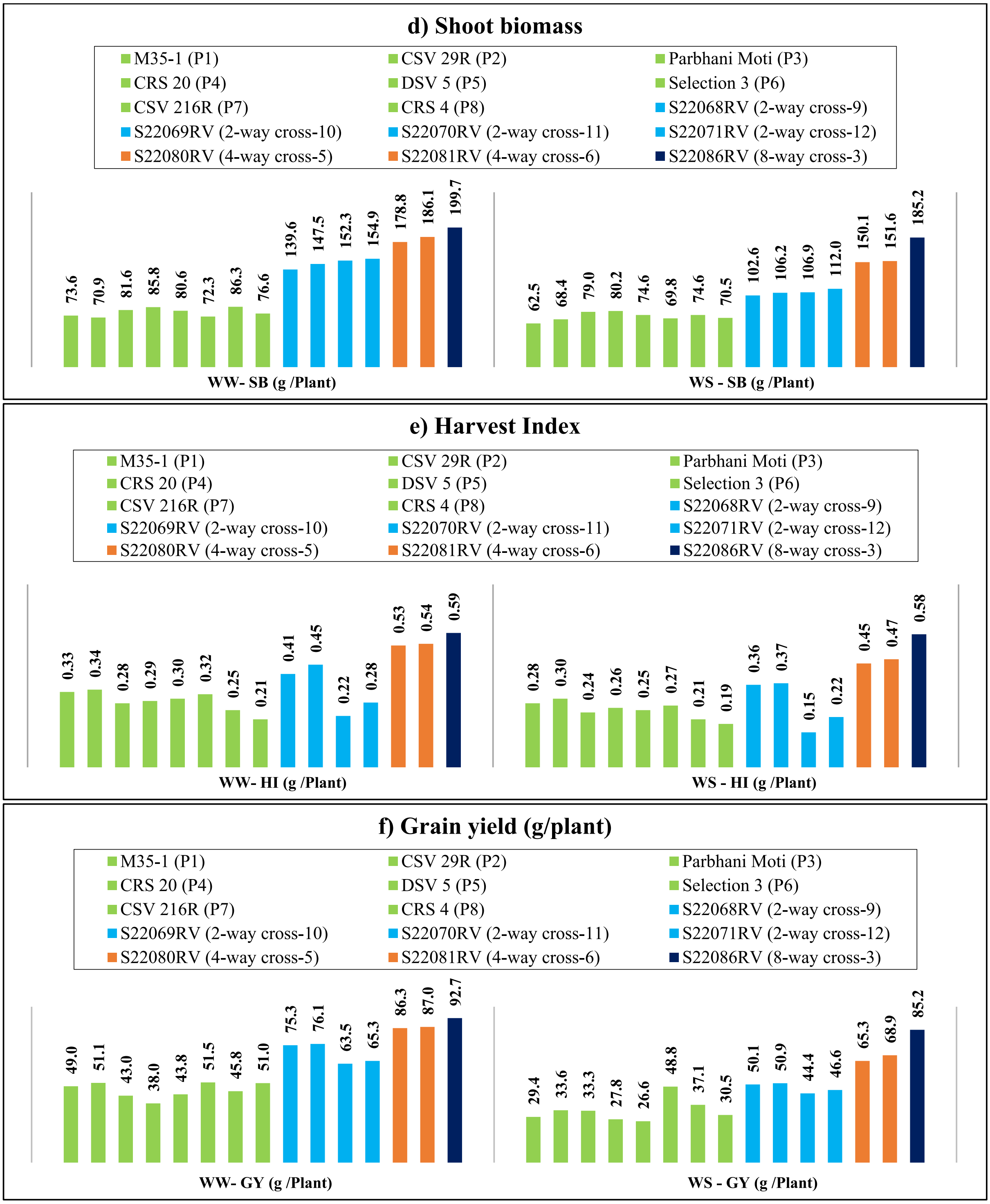


Fig. 6. Comparison of the performance of the best outstanding MPCD line, 8-way cross-3 (S22086RV), its corresponding 2-way and 4-way cross derivatives, as well as their respective founder genotypes for different water use component traits. The traits evaluated in this comparative analysis include; (**d**) shoot biomass, (**e**) harvest index, and (**f**) grain yield, assessed under both well-watered and water stress conditions in lysimetric studies.

sorghum biotic stresses in the MPCDs (eight-way cross) of post-rainy sorghum. Interestingly, while most of the 2-way and 4-way cross derived lines showed a moderately resistant reaction to the shoot fly, the 8-way and a few 4-way cross derived lines were recorded as resistant. The two of the MPCD lines SS22086RV (11.72% DH) and SS22087RV (12.43%) exhibited the lowest incidence of shoot fly making them excellent donor sources for incorporating shoot fly resistance in breeding programs.

Similarly, the lines SS22086RV and SS22087RV were found to be highly resistant to charcoal rot, with a charcoal rot index of less than 10, while all other lines, including the parents, were only moderately resistant. The enhanced resistance to shoot fly in the 8-way cross derived lines is attributed to the synergistic effects of their vigorous seedling growth, the presence of glossy leaf surface, and higher trichome density, as reported in previous studies[69,70]. Furthermore, the resistance to charcoal rot disease in the 8-way cross derived lines is associated with the stay-green trait, which in turn contributed to reduced lodging of the plants. Such 8-way cross derived lines, exhibiting combined resistance to both shoot fly and charcoal rot, can be effectively utilized in sorghum improvement programs to develop superior cultivars with enhanced biotic stress tolerance[70,71]. The multi-parent cross-derived lines identified in this study have exhibited far superior levels of resistance to major biotic stresses affecting post-rainy season sorghum cultivation, such as shoot fly and charcoal rot. These lines have surpassed the established benchmarks (shoot fly < 15% DH and charcoal rot < 10 CRI) and standards set for the registration of genetic stocks, demonstrating their exceptional potential to contribute to sorghum improvement efforts and serve as valuable resources for breeding programs targeting enhanced resistance to these critical constraints in the post-rainy season sorghum production system.

Limitations and future research directions

The multi-parent crossing strategy employed in this study focused primarily on utilizing the founder parents belonging to a single ecological type and race, specifically the durra sorghum race. While this targeted approach has been successful in generating high-performing and stress-resilient superior genotypes, the genetic diversity created by such narrow combinations is somewhat limited. To further broaden the genetic base and introduce a wider range of genetic variation, it would be beneficial to incorporate founder parents from additional sorghum races, such as kafir and caudatum, in future iterations of this multi-parent crossing strategy. Expanding the diversity of the parental pool could lead to the generation of progenies with an even more diverse array of favorable traits and enhanced overall performance. Though the outcomes of multi-parent crossing strategies can be quite impactful, implementing these approaches is not a simple endeavor. It is a time-consuming process that demands unwavering attention, specialized skill, and a high level of dedication to execute effectively and efficiently. The successful implementation of multi-parent crossing requires a thorough understanding of plant phenology, meticulous planning, and careful execution of the crosses. Generating diverse and high-performing genotypes through these strategies is a complex undertaking that requires a significant investment of time, effort, and expertise.

To fully evaluate the genetic worth and performance potential of the generated multi-parent cross-derived lines, a more rigorous and extensive phenotyping regimen across multiple diverse locations and environments is required. This comprehensive phenotyping effort would provide crucial insights into the lines' adaptability, stability, and expression of key agronomic and quality traits under varying conditions. Additionally, generating high-quality genotypic data for these multi-parent populations can be highly valuable in exploring them as Multiparent Advanced Generation Inter-Cross populations. Such genotypic data can facilitate in-depth quantitative trait locus mapping and identification, enabling a deeper understanding of the underlying genetic architecture responsible for the observed variations in the lines. The integration of robust phenotyping and genotyping approaches can yield valuable insights to guide the selection and advancement of the most promising and high-performing lines from these multi-parent crossing strategies.

Conclusion

This study demonstrated the use of a comprehensive multi-parent crossing strategy to combine desirable traits from the eight founder lines and develop dual-purpose, high-yielding genotypes with improved tolerance to drought, shoot fly, and charcoal rot in post-rainy season sorghum. The systematic evaluation of advanced-generation derivatives from 8-way, 4-way, and 2-way crosses led to the identification of several promising lines exhibiting remarkably higher grain and stover yields, up to 60–70% and 15–30% respectively, compared to the existing national check cultivar CSV 29R. The lines, *SS22086RV,* a derivative from the 8-way cross [(M35-1 × CSV 29R) × (Parbhani Moti × CRS 20)] × [(DSV 5 × Selection 3) × (CSV 216R × CRS4)] and *SS22085RV,* a derivative from the cross [(PKV Kranti × Solapur Dagadi) × (Phule Suchitra × CSV 14R)] × [(M 35-1 × CSV 29R) × (CSV 216R × CRS 4)] displayed excellent agronomic attributes, such as bold seeds, long and compact panicles, and better peduncle length and panicle exertion. The medium maturity and medium-tall plant height of these multi-parent cross-derived lines further enhance their suitability for post-rainy season cultivation. Importantly, these MPCD lines possess key water use efficiency traits, conferring enhanced drought tolerance, as well as remarkably low incidences of shoot fly (< 15% DH) and charcoal rot (< 10 CRI), making them valuable genetic resources for stress resistance breeding. These MPCD lines can be further evaluated through multi-location trials under the national coordinated research program for the isolation of superior varieties. The diverse and well-adapted multi-parent cross-derived lines developed in this study serve as valuable genetic resources that can be leveraged for genomic selection strategies to dissect the key adaptive traits required for successful post-rainy season cultivation. These tailor-made genotypes and populations exhibit a rich genetic diversity that can be exploited to identify quantitative trait loci and genomic regions associated with critical post-rainy season adaptation mechanisms.

Data availability

The original data presented in this study are available in the article and supplementary file itself. Readers who require further information or wish to access the underlying data can contact the corresponding author for assistance. The authors welcome inquiries and requests related to the data gathered and analyzed in this research work.

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References

1. Marchini, M. et al. Sprouting of Sorghum (*Sorghum bicolor* [L.] Moench): Effect of drying treatment on protein and starch features. *Foods.* **10**, 407. https://doi.org/10.3390/foods10020407 (2021).
2. Baloch, F. S. et al. Recent advancements in the breeding of sorghum crop: current status and future strategies for marker-assisted breeding. *Front. Genet.* **14**, 1150616. https://doi.org/10.3389/fgene.2023.1150616 (2023).
3. Yedemie, S. Promotion of improved management of sorghum for small scale farmers in north gondar, gondar zuria district. *Crimson Publ.* **2**, 128–130. https://doi.org/10.31031/mcda.2018.02.000533 (2018).
4. Dhutmal, R., More, A. & Ayewar, N. Current status and future prospects of millets with special reference to sorghum (*Sorghum bicolor* Moench L). *AkiNik Publ.* **8**, 2493–2499. https://doi.org/10.22271/chemi.2020.v8.i6aj.11146 (2020).
5. Rooney, W. L. Sorghum: Production and Improvement Practices. https://www.sciencedirect.com/science/article/pii/B9780123944375001984 (2023).
6. Pawar, S. E. & Gadakh, S. R. Behavior of sorghum cultivars under decreasing levels of soil moisture condition. *Excellent Publ.* **7**, 1906–1913. https://doi.org/10.20546/ijcmas.2018.709.231 (2018).
7. Kholova, J., McLean, G., Vadez, V., Craufurd, P. & Hammer, G. Drought stress characterization of post-rainy season (rabi) sorghum in India. *Field Crops Res.* **141**, 38–46. https://doi.org/10.1016/j.fcr.2012.10.020 (2013).
8. Sharma, K. L. et al. Strategies for improving the productivity of rainfed farms in India with special emphasis on soil quality improvement. *J. Crop Improv.* **23**, 430. https://doi.org/10.1080/15427520903013431 (2009).
9. Das, I. K. & Rajendrakumar, P. Disease resistance in sorghum. https://www.sciencedirect.com/science/article/pii/B9780128045497000020 (2016).
10. Patil, J. V., Reddy, P. S., Prabhakar, U. A. V., Gomashe, S. & Ganapathy, K. N. History of post-rainy season sorghum research in India and strategies for breaking the yield plateau. *Indian J. Genet.* **74**, 271–285 (2014).
11. Kumar, M. V. N. et al. India's rainfed sorghum improvement: Three decades of genetic gain assessment for yield, grain quality, grain mold and shoot fly resistance. *Front. Plant Sci.* **13**, 1056040. https://doi.org/10.3389/fpls.2022.1056040 (2022).
12. Parashuram P. et al. Early generation testing and advancement of selected multi-parent cross derived genotypes of post rainy sorghum (*Sorghum bicolor* L. Moench). In *1st International Symposium on "Cereals for Food Security and Climate Resilience" organized by ICAR-IIWBR, Karnal* (2022).
13. Parashuram P. et al. Advanced generation multi-parent crossing for broadening genetic base of grain yield traits in Rabi Sorghum [(*Sorghum bicolor* L.) Moench]". In *International Conference on Advances in Agriculture & Allied Science Research, Kanpur.* 159. (2019).
14. Kholová, J. et al. Water saving traits co-map with a major terminal drought tolerance quantitative trait loci in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Mol. Breed.* **30**, 1337–1353 (2012).
15. Adotey, R. E. et al. Water-deficit stress alters intra-panicle grain number in sorghum. *Crop Sci.* **61**, 2680. https://doi.org/10.1002/csc2.20532 (2021).
16. Khandelwal, V., Keerthika, A., & Dhoot, M. Stability Analysis for Yield and Component Traits in Sorghum [*Sorghum bicolor* (l.) Moench.]. *Int. J. Curr. Microbiol. Appl. Sci.* **8,** 1910. https://doi.org/10.20546/ijcmas.2019.809.221 (2019).
17. Reddy, P. S. Breeding for abiotic stress resistance in Sorghum. In *Elsevier eBooks*, vol. 325 (ed. Reddy, P. S.) https://doi.org/10.1016/b978-0-08-101879-8.00020-6 (Elsevier BV, 2018).
18 Shivakumar, M., Kumawat, G., Gireesh, C., Ramesh, S. V. & Husain, S. M. Soybean MAGIC population: a novel resource for genetics and plant breeding. *Curr. Sci.* **114**, 906–908. https://doi.org/10.18520/cs/v114/i04/906-908 (2017).
19 Upadhyaya, H. D. et al. Multi-trait diverse germplasm sources from mini core collection for sorghum improvement. *Multidiscip. Digit. Publ. Inst.* **9**, 121–121. https://doi.org/10.3390/agriculture9060121 (2019).
20 Koli, N. R., Patidar, B. K., Kumar, M. & Dhya, S. Biparental mating: A system of intermating for creating genetic variability in segregating generation for crop improvement. *Int. J. Curr. Microbiol. Appl. Sci.* **7**, 3592. https://doi.org/10.20546/ijcmas.2018.708.363 (2018).
21. Huang, J. et al. RAD18 transmits DNA damage signalling to elicit homologous recombination repair. *Nat. Cell Biol.* **11**, 592–603. https://doi.org/10.1038/ncb1865 (2009).
22. Klasen, J. R., Piepho, H. & Stich, B. QTL detection power of multi-parental RIL populations in *Arabidopsis thaliana*. *Heredity* **108**, 626. https://doi.org/10.1038/hdy.2011.133 (2012).
23. Scott, M. F. et al. Multi-parent populations in crops: a toolbox integrating genomics and genetic mapping with breeding. *Heredity* **125**, 396–416. https://doi.org/10.1038/s41437-020-0336-6 (2020).
24 Razzaq, A., Kaur, P., Akhter, N., Wani, S. H. & Saleem, F. Next-generation breeding strategies for climate-ready crops. *Front. Plant Sci.* **12**, 620420. https://doi.org/10.3389/fpls.2021.620420 (2021).
25. Harlan, H. & Martini, M. A composite hybrid mixture. *Agron. J.* **21**, 487–490 (1929).
26. Suneson, C. A. An evolutionary plant breeding method. *Agron. J.* **48**, 188–191 (1956).
27. Eberhart, S. Techniques and methods for more efficient population improvement in sorghum. In *Sorghum in Seventies* (eds. Rao, N., & House, L.) 197–213 (Oxford and IBH Publishing Co., 1972).
28. Reddy, B. V. S. & Stenhouse, J. W. Sorghum improvement for semi-arid tropics region: past, current and future research thrusts in Asia. *PKV Res. J.* **18**, 155–169 (1994).
29. Stadlmeier, M., Hartl, L. & Mohler, V. Usefulness of a multiparent advanced generation intercross population with a greatly reduced mating design for genetic studies in winter wheat. *Front. Plant Sci.* **9**, 1825. https://doi.org/10.3389/fpls.2018.01825 (2018).
30. Odell, S. G. et al. Modelling allelic diversity of multiparent mapping populations affects detection of quantitative trait loci. *G3 (Bethesda)*. https://doi.org/10.1093/g3journal/jkac011 (2022)
31. Arrones, A. et al. The dawn of the age of multi-parent MAGIC populations in plant breeding: novel powerful next-generation resources for genetic analysis and selection of recombinant elite material. *Biology (Basel)*. **16,** 229. https://doi.org/10.3390/biology9080229 (2020).
32. Huang, B.E. *et al.* A multiparent advanced generation inter-cross population for genetic analysis in wheat. *Plant Biotechnol. J.* **10,** 826–839. https://doi.org/10.1111/j.1467-7652.2012.00702.x (2012).
33. Mackay, I.J. *et al.* An eight-parent multiparent advanced generation inter-cross population for winter-sown wheat: creation, properties, and validation. G3 (Bethesda). **4**, 1603–1610 (2014).
34. Bandillo, N. et al. Multi-parent advanced generation inter-cross (MAGIC) populations in rice: progress and potential for genetics research and breeding. *Rice.* **6**, 11 (2013).
35. Pascual, L. et al. Potential of a tomato MAGIC population to decipher the genetic control of quantitative traits and detect causal variants in the resequencing era. *Plant Biotechnol. J.* **13**, 565–577. https://doi.org/10.1111/pbi.12282 (2015).
36. Sallam, A. & Martsch, R. Association mapping for frost tolerance using multi-parent advanced generation inter-cross (MAGIC) population in faba bean (*Vicia faba* L.). *Genetica* **143**, 501–514. https://doi.org/10.1007/s10709-015-9848-z (2015).
37. Dell'Acqua, M., Gatti, D. & Pea, G. Genetic properties of the MAGIC maize population: a new platform for high definition QTL mapping in *Zea mays*. *Genome Biol.* **16**, 1–23 (2015).

38. Anderson, S. L., Mahan, A. L., Murray, S. C. & Klein, P. E. Four parent maize (FPM) population: effects of mating designs on linkage disequilibrium and mapping quantitative traits. *Plant Genom.* **11**, 1–17. https://doi.org/10.3835/plantgenome2017.11.0102 (2018).
39. Sannemann, W., Huang, B. E., Mathew, B. & Léon, J. Multi-parent advanced generation inter-cross in barley: high-resolution quantitative trait locus mapping for flowering time as a proof of concept. *Mol. Breed.* **35**, 1–6. https://doi.org/10.1007/s11032-015-0284-7 (2015).
40. Ongom, P. O. & Ejeta, G. Mating design and genetic structure of a multi- parent advanced generation intercross (MAGIC) population of sorghum (Sorghum bicolor L. Moench). *G3 (Bethesda) Genes|Genomes|Genetics*. **8,** 331–341. https://doi.org/10.1534/g3.117.300248 (2018).
41. Neeraj Kumar et al. Development and characterization of a sorghum multi- parent advanced generation intercross (MAGIC) population for capturing diversity among seed parent gene pool. *G3 Genes|Genomes|Genetics*. https://doi.org/10.1093/g3journal/jkad037 (2023).
42. Hashemi, S. M., Perry, G., Rajcan, I. & Eskandari M. SoyMAGIC: An unprecedented platform for genetic studies and breeding activities in soybean. *Front. Plant Sci.* https://doi.org/10.3389/fpls.2022.945471 (2022).
43. Hariprasanna, K. & Patil, J. V. Sorghum: origin, classification, biology and improvement. In *Sorghum Molecular Breeding* (eds Madhusudhana, R., Rajendra Kumar, P. & Patil, J. V.) 3–20 (Springer (India) Pvt. Ltd., 2015).
44. Patterson, H. & Williams, E. A new class of resolvable incomplete block designs. *Biometrika* **63**, 83–92. https://doi.org/10.1093/biomet/63.1.83 (1976).
45. Sanchez M. J. et al. Varietal descriptors of sorghum varieties (Sorghum bicolor L. Moench) for registration and breeder's rights. *J.-Agrarian Nat. Resour. Econ.* **4**, 1–5 (2020).
46. Passioura, J. B. Grain yield, harvest index and water use of wheat. *J. Aust. I Agric. Sci.* **43**, 117–120 (1977).
47. Taneja, S.L. & Leuschner, K. Resistance screening and mechanisms of resistance in sorghum to shoot fly. In *Proceedings of the International Sorghum Entomology Workshop, Texas, USA* 115–112 (International Crops Research Institute for the Semi-Arid Tropics, 1985).
48. Nimbalkar, V. S. & Bapat, D. R. Genetic analysis of shoot fly resistance under high level of shoot fly Infestation in Sorghum. *J. Maharashtra Agric. Univ.* **12**, 331–334 (1987).
49. Kamatar, M. Y., Patil, A. M., Yadwab, A., Salimath, P. M. & Rao, T. S. Correlation and path analysis in parents and hybrids for resistance to sorghum shoot fly [*Atherigona soccata* (Rondani)]. *Int. J. Plant Sci.* **5**, 399–403 (2010).
50. Das, I. K. et al. Development of a charcoal rot rating index for multilocation trials sorghum. *Crop Prot.* **108**, 102–109 (2018).
51. Patterson, H. & Williams E. A new class of resolvable incomplete block designs. *Biometrika*. **63**, 83–92. https://doi.org/10.1093/biomet/63.1.83 (1976)
52. Gopinath, P. P., Parsad, R., Joseph, B. & Adarsh, V. S. grapesAgri1: Collection of shiny apps for data analysis in agriculture. *J. Open Source Softw.* **6**, 3437 (2021).
53. Nitesh, S.D., Parashuram, Patroti & Shilpa Parashuram. TraitStats: Analysis of RCBD data for descriptive statistics, ANOVA, genetic parameters, correlation, and covariance. *R package version* 1.0.0 (2020).
54. Parthasarathy Rao, P. & Basavaraj, G. Status and prospects of millet utilization in India and global scenario. In: *Millets: Promotion for Food, Feed, Fodder, Nutritional and Environment Security, Proceedings of Global Consultation on Millets Promotion for Health & Nutritional Security*, 197–209 (Society for Millets Research, ICAR Indian Institute of Millets Research, 2015).
55. Jahufer, M. & Casler, M. Application of the Smith-Hazel selection index for improving biomass yield and quality of switchgrass. *Crop Sci.* **55**, 1212–1222. https://doi.org/10.2135/cropsci2014.08.0575 (2015).
56 Varshney, R. K., Graner, A. & Sorrells, M. E. Genomics-assisted breeding for crop improvement. *Trends Plant Sci.* **10**, 621–630. https://doi.org/10.1016/j.tplants.2005.10.004 (2005).
57. Zheng, C. Modeling X-Linked ancestral origins in multi-parental Populations. *G3 (Bethesda).* **5,** 777–801 (2015)
58. Diaz, S., Ariza-Suarez, D., Izquierdo, P. et al. Genetic mapping for agronomic traits in a MAGIC population of common bean (*Phaseolus vulgaris* L.) under drought conditions. *BMC Genom.* **21,** 799 https://doi.org/10.1186/s12864-020-07213-6 (2020).
59. Verbyla, A. P., Cavanagh, C. R. & Verbyla, K. L. Whole-genome analysis of multi-environment or multi trait QTL in MAGIC. *G3 (Bethesda).* **4,** 1569–1584 (2014).
60. Huang, B. E. et al. MAGIC populations in crops: current status and future prospects. *Theor. Appl. Genet.* **128**, 999–1017 (2015).
61. Holland, J. MAGIC maize: a new resource for plant genetics. *Genome Biol.* **16**, 163 (2015).
62. Parashuram, P. et al. Identification of rabi (post rainy season) sorghum varieties with enhanced levels of drought tolerance using component traits of water use. In *National Seminar on Millets, organized by Society for Agricultural Research on Abiotic Stress (SARAS) & ICAR-National Institute of Abiotic Stress Management, Baramati*. Theme I-36-P, 22 (2023).
63. Parashuram, P. et al. Assessment of Multi-parent cross induced genetic variability in the segregating generations (F2s) of post rainy season sorghum. In *Presented in the Tending Mendel's Garden for a Perpetual and Bountiful Harvest: Symposium Commemorating Birth Bicentenary of Gregor Johann Mendel (MENDELSYM), New Delhi, India* (2022).
64. Archana, B. et al. Study of variability in F2 Derived F3 populations of multi-parent crosses in rabi sorghum under rainfed situation. In *National Seminar on Millets, organized by Society for Agricultural Research on Abiotic Stress (SARAS) & ICAR-National Institute of Abiotic Stress Management, Baramati*. Theme I-44-P, 28 (2023).
65. Abreha, K. B. et al. Sorghum in dryland: morphological, physiological, and molecular responses of sorghum under drought stress. *Planta* **255**, 20. https://doi.org/10.1007/s00425-021-03799-7 (2022).
66. Mutisya, J., Sitieney, J. K. & Gichuki, S. T. Phenotypic and physiological aspects related to drought tolerance in sorghum. *Afr. Crop Sci. J.* **18**, 175–182 (2010).
67. Debabrata, P., Swati S. M, Behera P. K. Drought tolerance in rice: focus on recent mechanisms and approaches. *Rice Sci.* **28**, 119–132. https://doi.org/10.1016/j.rsci.2021.01.002 (2021).
68. Samdur, M. Y. *et al.* Weighted geometric mean index: A model to evaluate drought tolerance in post-rainy season sorghum (Sorghum bicolor (L.) Moench). *Cereal Res. Commun*. **49,** 329–336 (2020).
69. Kishore, P. Breeding for resistance to shoot fly and stem-borer in sorghum. *Springer Nat.* **7**, 117–121 (1986).
70. Dhillon, M. K., Sharma, H. C., Singh, R. & Naresh, J. S. Mechanisms of resistance to shoot fly, *Atherigona soccata* in sorghum. *Springer science + Business media*. **144,** 301–312 (2005).
71. Syed, A., More, A W. & Kalpande, H V. Character association studies in sorghum [*Sorghum bicolor* (L.) Moench] germplasm lines for shoot fly resistance parameters. *Excellent Publ.* **6,** 298–302. https://doi.org/10.20546/ijcmas.2017.612.035 (2017).

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Author contributions

P.P. conceptualized and designed the study, and wrote the manuscript; R.M. contributed by reviewing and edit-

ing the manuscript; S.S. carried out the drought screening studies; G.S. conducted the shoot fly screening; B.R. and I.D. performed the charcoal rot screening; C.T.S. provided support in the form of institute funds and field facilities. All authors have read and agreed to the final version of the manuscript.

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Competing interests
The authors declare no competing interests.

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