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RESEARCH ARTICLE

Introgression and evaluation of glyphosate tolerance gene *EPSPS* into the Indian soybean (*Glycine max* L.) variety JS9560 through marker assisted backcross breeding

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Abstract

Weeds are becoming increasingly prevalent, which poses a serious threat to soybean production in India and results in significant yield losses. Developing herbicide-tolerant soybean cultivars, when coupled with a lack of novel herbicides, increases the herbicidal spectrum and reduces crop phytotoxicity, making it an efficient weed management method. Currently, no Indian soybean is herbicide-tolerant, and no study on the development of herbicide-tolerant soybeans has been executed in India. Introgression of an herbicide-tolerant gene into the genetic background of popular Indian soybean is an effective strategy to develop novel soybean cultivars. Therefore, the molecular marker-assisted backcross breeding (MABB) approach was adopted in the current study for the genetic introgression of a glyphosate-tolerant gene, *EPSPS*, to develop herbicide-tolerant soybean. The introgression of the glyphosate-tolerant gene, *EPSPS*, from an American herbicide-tolerant soybean variety (S14-9017GT) into the genetic background of the elite popular Indian soybean variety, JS 9560 (the recurrent parent), was done using MABB. Foreground selection was done using the *EPSPS* gene-linked Simple Sequence Repeat (SSR) marker EPSPS2, and background selection was performed using 124 genome-wide SSR markers polymorphic between S14-9017GT and JS 9560. Selection of glyphosate-Tolerant lines in each generation until BC_2F_2 was carried out to improve the recovery of the recurrent parent genome (RPG). About 38 BC_2F_2 plants were developed and found to recover 94.35 to 98.77% of the recurrent parent genome. The improved BC_2F_2 population was evaluated for herbicide tolerant using glyphosate spray (3 ml/litre), and it was found that fourteen (14) BC_2F_2 plants were homozygous (*EPSPS/EPSPS*) for the glyphosate Tolerant trait, in which P-11-09 is showing the highest recurrent parent genome recovery (98.77%). Selfing of positive BC_2F_2 plants resulted in 583 BC_2F_3 population. Phenotypic selection for agronomic traits in the Tolerant BC_2F_3 generation was carried out, and it was recorded that the performance of BC_2F_3 plants was like that of the recurrent parent, JS 9560. Overall, the study showed that the glyphosate-tolerant *EPSPS* allele was successfully introgressed into the genetic background of the popular soybean variety JS 9560, resulting in the development of herbicide-tolerant (HT) BC_2F_3 lines. This is the first study on the breeding of herbicide-tolerant soybeans in India. The superior plants developed will be tested in further breeding programs to develop soybean cultivars with glyphosate tolerant in India.

Keywords: Marker assisted backcross breeding, *EPSPS*, glyphosate tolerant, foreground and background selection, agronomic traits, soybean.

Introduction

Improving essential crops, such as cereals, legumes, and oilseeds has been a continuous effort for agricultural improvement. The soybean (*Glycine max* L. Merrill) is undoubtedly one of the most significant crops in the world due to its wide variety of applications (food, oilbiodiesel, secondary metabolites, etc.) and the high market value of its products (Sharma et al. 2011). It is among the most important crops in the world for oil and proteins (Zhang et al. 2022). According to Duan et al. (2023), seeds from modern soybean cultivars have a protein content of 35% and an oil content of about 17%. The global domestic consumption of soybean oil is estimated to be 65,273 thousand metric tonnes (USDA-FAS 2024). The soybean is now an essential part of global trade and agronomic systems due to its many potential uses. USA, Brazil, and Argentina contributed a projected 314.48 million metric tonnes of soybeans in 2023, accounting for 79% of global production (USDA 2024).

Enhancing soybean yields and maintaining a high-quality harvest free of weed seeds require effective weed management (Padgette et al. 1995). Weeds can easily disrupt soybeans because the seeds are spaced widely apart to create branches and enable the canopy to fully emerge later in the growth stage. Compared to other crops, soybeans are more vulnerable to weeds due to their prolonged canopy closure (Song et al. 2020). When it comes to soybean production, weeds are the biggest source of yield loss.

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Evidence suggests that weed competition threatens 37% of soybean yields worldwide (Kanatas et al. 2020).

Herbicides are being widely used in soybean cultivation to eradicate weeds. The most efficient way to control weeds is frequently to spray chemical herbicides, either selective or non-selective. Nonetheless, inappropriate herbicide application can lead to major concerns such crop loss and the emergence of herbicide-tolerant weeds (Mackelprang et al. 2020). Therefore, developing cultivars tolerant to herbicides provides an effective solution. (Hussain et al. 2021; Dong et al. 2021), which can lower the cost of weeding (Green 2012), increase the herbicidal spectrum (Shaner 2000), and decrease crop phytotoxicity caused by herbicide treatment (Glick, 2001), thereby reducing weed growth and significantly boosting crop yield (Kumar et al. 2008).

In field crops, only specific weeds are killed with selective herbicides, which safeguard desirable plants. Farmers must, however, invest time in determining the different types of weeds and creating control strategies for them (Dill et al. 2008; Green et al. 2011, 2014). On the other hand, most plants, including weeds and crops, are killed by broad-spectrum or non-selective herbicides. Glyphosate is the most used non-selective herbicide worldwide. According to Tan et al. (2006), glyphosate works by blocking 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an essential enzyme in the shikimate pathway that is involved in the synthesis of aromatic amino acids. Glyphosate-tolerant (GR) soybeans were among the first major applications of genetic engineering (Elmore et al. 2001). The glyphosate-tolerant clone CP4-EPSPS was introduced into plants to produce glyphosate-tolerant crops. This transgene makes it possible for plants to withstand glyphosate treatment by allowing the shikimate pathway to function in the presence of glyphosate. This enzyme is rendered insensitive to glyphosate by a genetic modification identified in glyphosate-tolerant plants (Funke et al. 2009).

Several studies have recently been reported on the use of various modern methods of plant breeding for developing glyphosate-tolerant soybean cultivars (Niu et al. 2024; Wei et al. 2023; Guoet al. 2020; Xiao et al. 2019). However, so far, no Indian soybean variety has developed tolerant to the herbicide glyphosate. Currently, no genetic research has been done on Indian soybeans that have the EPSPS transgene governing glyphosate tolerant. Thus, the objective of the study was to create glyphosate-tolerant soybeans by marker-assisted backcross breeding (MABB) through the transfer of the herbicide tolerant gene, *EPSPS*, from an American herbicide-tolerantsoybean variety (S14-9017GT) to Indian soybean variety (JS 9560). Monsanto Co. created and commercialized the transgene (EPSPS), but it is now off-patent and can be used for commercial applications without any intellectual property constraints.

Materials and methods

Plant materials

In this study, we used the glyphosate tolerant soybean cultivar S14-9017GT as donor parental line and JS9560 as the recurrent parent (RP). The donor is a high yielding (43.14 q/ha) variety with a duration of 145-155 days. The recurrent parent JS 9560 is a local early duration (88-90 days) variety with yield potential of 18-20 q/ha. It was obtained from the Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh. The research experiment was conducted in the glasshouse (Fig. 1) at the National Phytotron Facility, Indian Agricultural Research Institute (IARI), New Delhi.

MABB strategy for development of herbicide tolerant lines

The tolerant donor line S14-9017GT and the recurrent variety JS 9560 were initially crossed in 2021 to produce

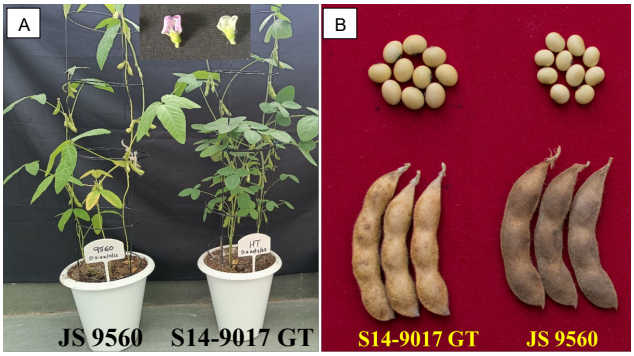

Fig. 1. Plant materials used in the experiment. A. Parental lines showing contrasting characters viz., plant height, leaf shape, Flower colour and maturity B. Pod and seed weight variation of parental lines grown in glasshouse in *kharif* 2023

F_1 seeds. The hybridity of F_1 plants was tested using the SSR marker Satt543, which is linked to the *EPSPS* gene. The BC_1F_1 seeds were generated by backcrossing the true F_1s with JS 9560. In *kharif* 2022, BC_1F_1 plants of the JS 9560 population were grown and their genetic background was examined for tolerant genes. To produce the BC_2F_1 seeds, positive BC_1F_1 plants were backcrossed to JS 9560. In each generation, the plant heterozygous for the *EPSPS* allele and the highest recovery for Recurrent Parent Genome (RPG) and Recurrent Parent Phenome (RPP) were identified using a similar approach up to the BC_2F_3 generation. The superior BC_2F_1 plants were advanced to BC_2F_2 generation and plants homozygous for the EPSPS allele were identified. Further, the selected BC_2F_2 plants were advanced to BC_2F_3 generation via plant to progeny selection.

Molecular analysis

DNA was extracted using the CTAB method developed by Doyle and Doyle (1990). A polymorphism study was carried out using more than 400 SSR markers that are distributed across the genome. A total of 10 µl of PCR was prepared, containing 2X red dye PCR master mix (Genei Laboratories Pvt. Ltd. Bangalore), 5 pmol of each forward and reverse primer, and 25–30 ng of template DNA. Initial denaturation at 95 °C for 5 minutes, 35 cycles of denaturation at 95 °C for 40 s, annealing at 58 °C for 40 s, extension at 72 °C for 1 minute, and a final extension at 72 °C for 10 minutes comprised the PCR amplification procedure. The amplified products were resolved on 3.5% agarose gel mixed with 0.1 mg/ml ethidium bromide. The amplicons were visualized on ultraviolet trans-illuminator (Gel Doc™ + Imager, Bio- Rad Laboratories Inc., U.S.A).

Foreground and background selection

Using the SSR marker EPSPS2 linked with the *EPSPS* gene at 1.2cM in chromosome 2, foreground selection was performed to identify plants carrying the herbicide tolerant allele. For background selection, 124 polymorphic SSR markers between the parents JS 9560 and S14-9017GT were identified employing a total of 485 SSR markers (Table 1). At each marker locus, the homozygous and heterozygous plants for the JS 9560 allele were counted independently throughout the background selection in backcross generations. Markers that were identified to be homozygous for the JS 9560 allele in each generation were excluded from subsequent generations for background selection, using a reductionist approach. The formula was used to estimate RPG recovery.

$$\text{RPG recovery \%} = \frac{\text{No. of markers homozygous for RP alleles + (0.5)x No. of heterozygous markers}}{\text{Total number of polymoprhic markers used for background selection}} \times 100$$

The RPG recovery was visualized using Graphical GenoTypes (GGT) Version 2.0 software (Van Berloo 1999).

Screening for Glyphosate Tolerant lines

In plant-to-progeny rows, thirty-eight (38) BC_2F_2 plants of JS 9560,and S14-9017GT were sprayed with glyphosate (marketed as Roundup), at a dosage of 3 ml per litre. Using the conventional methodology for soybeans, a visual assessment of the BC_2F_3's herbicide tolerance was performed 15 days after the spray.

Evaluation of agronomic traits

The improved tolerant BC_2F_3 plants and the parents were agro-morphologically evaluated in glasshouse conditions following recommended agronomic practices. Data on plant height (cm), number of pods per plant, number of seeds per plant, 100 seed weight, and seed yield per plant were collected, along with information on yield and yield-related characteristics. CropStat 7.2 was used to do the statistical analysis of the agro-morphological data.

Results

In MABB, the *EPSPS* gene was transferred from donor parent, S14-9017GT into the genetic background of recurrent parent JS9560. A SSR marker called Satt543 was used to confirm the hybridity of the F_1 plants derived from the cross between JS9560 and S14-9017GT. Following a backcross to the respective parents, 68 BC_1F_1 plants were produced. These 68 plants were subjected to foreground selection (Fig. 2) using the gene-linked SSR marker *EPSPS*2, which revealed that 32 plants were heterozygous for *EPSPS* genes. A set of 65 BC_2F_1 plants were produced by backcrossing plants with the highest RPG and the highest degree of phenotypic resemblance to JS9560. After 65 plants were subjected to foreground selection, 30 BC_2F_1 plants tested positive for the

Table 1. Number of plants generated in the backcross generations during marker aidedintrogression of EPSPS allele in JS 9560

Generations	Total number of seeds	Number of plants obtained	No of positive plants	No. of homozygous plants
F_1	124	86	75	-
BC_1F_1	70	68	32	-
BC_2F_1	72	65	30	-
BC_2F_2	46	38	30	14
BC_2F_3	605	583	455	-

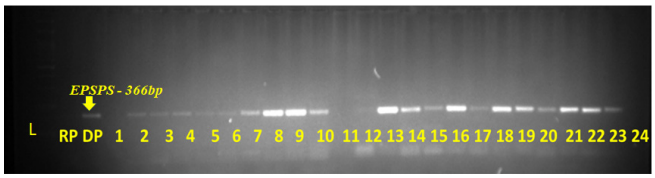

Fig. 2. A representative gel image showing the amplification profile of *EPSPS* gene linked marker Satt543 in the F_1 hybrids along with recurrent parent (RP), JS 9560 and donor parent (DP)S14-9017GT. M: 100 base pair DNA ladder, DP: S14-9017GT, RP: JS 9560

EPSPS gene. After being selfed, the positive plants produced 30 BC_2F_2 plants (Table 1).

The 38 BC_2F_2 plants were subjected to foreground selection using EPSPS gene-linked SSR markers. Thirty plants tested positive for the herbicide tolerant gene. Using 124 polymorphic SSR markers, the positive BC_2F_2 plants were once more selected for background selection. With an average recovery of 96.5%, the recurrent parent genome recovery percentage in the BC_2F_2 generation varied from 94.35 (P-1-6 and P-24-2) to 98.77% (P-11-9) (Table 2). All plants had some residual donor elements in most of their chromosomes; however plant P-11-9 had the greatest recurring parent genome recovery percentage (98.77%) (Fig. 3). The phenotypic performance of glyphosate tolerance was assessed in the BC_2F_3 plants that tested positive.

Screening of BC_2F_2 plants for tolerance to herbicide glyphosate

Plant-to-progeny rows were used for growing the selected BC_2F_2 plants. Phenotypic screening of BC_2F_2 plants along with Recurrent Parent (RP), JS 9560 and Donor Parent, S14-9017GT for Glyphosate tolerance, was done by application of glyphosate @ 3 ml/liter (Fig. 4). Out of all the plants that were examined, there were about 14 which were homozygous (EPSPS/EPSPS) for the herbicide tolerant trait and 16 that were heterozygous (*EPSPS/epsps*) for the gene. Eight plants, however, showed a recessive homozygous form (epsps/epsps) for the trait that was found to be susceptible to herbicide application. In homozygous tolerant BC_2F_2 plants, the degree of herbicide tolerant was like that of the donor S14-9017GT (Fig. 5).

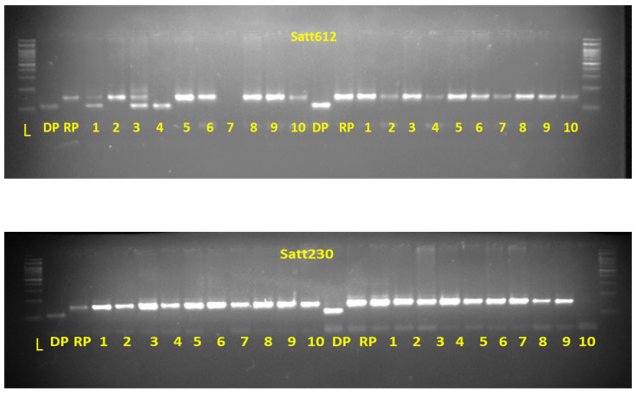

Fig. 3. A representative gel image showing the amplification profile of *EPSPS* gene linked marker *EPSPS*2 in the BC_2F_2 plants in background selection along with recurrent parent (RP), JS 9560 and donor parent (DP)S14-9017GT. M: 100 base pair DNA ladder, DP: S14-9017GT, RP: JS 9560

After harvesting the EPSPS homozygous tolerant BC_2F_2 plants, 605 BC_2F_3 seeds were produced, resulting in 583 plants. Of these, 455 plants tested positive for the EPSPS gene. The plants were then evaluated for phenotypic expression of yield and yield-contributing traits, such as plant height (cm), days to flowering, days to harvest, number

Table 2. Recovery of Recurrent Parent Genome (RPG) estimation in 30 BC_2F_2 (JS 9560) lines

S. No.	BC_2F_2 line	A	H	B	RPG % (A+H/2)/N
1	P-1-3	118	4	2	96.77
2	P-1-6	114	6	4	94.35
3	P-1-12	120	4	0	98.40
4	P-1-14	116	5	3	95.56
5	P-1-15	122	1	1	98.40
6	P-4-3	116	4	4	95.16
7	P-4-8	119	2	3	96.77
8	P-4-10	115	6	3	95.16
9	P-6-1	118	4	2	95.16
10	P-6-2	120	2	2	97.61
11	P-6-3	115	8	1	95.98
12	P-6-4	118	4	2	95.16
13	P-6-5	120	2	2	97.61
14	P-11-1	116	5	3	95.56
15	P-11-2	118	7	2	97.98
16	P-11-4	117	4	3	95.96
17	P-11-8	119	4	1	97.58
18	P-11-9	122	3	0	98.77
19	P-11-16	121	2	1	98.39
20	P-11-18	118	4	2	96.77
21	P-12-1	117	3	4	95.56
22	P-12-2	115	7	2	95.56
23	P-12-3	118	5	2	97.17
24	P-24-2	113	8	3	94.35
25	P-24-5	117	4	3	94.96
26	P-24-7	117	4	3	95.96
27	P-24-8	119	2	3	96.77
28	P-31-4	115	6	3	95.16
29	P-31-6	115	7	2	95.56
30	P-31-11	117	4	3	95.96

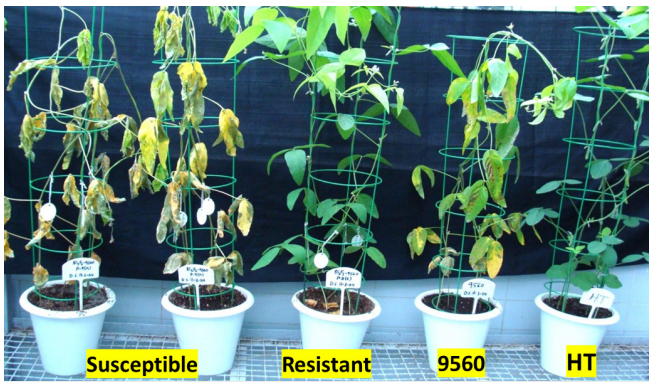

Fig. 4. Phenotypic screening of JS 9560 BC_2F_2 along with Recurrent Parent (RP), JS 9560 and Donor Parent, S14-9017GT for Tolerant to the herbicide, Glyphosate, application of glyphosate @ 3 ml/liter

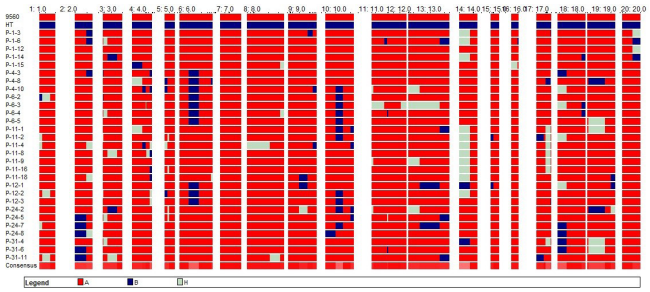

Fig. 5. Graphical representation of JS 9560 BC_2F_2 carrying mutant EPSPS gene showing the extent of recurrent parent genome (RPG) recovery

of pods per plant, number of seeds per plant, 100 seed weight, and seed yield per plant. Data on yield and traits related to yield was documented.

Evaluation of BC_2F_3 glyphosate tolerant plants for yield and yield contributing traits

The mean performance of BC_2F_3 plants along with recurrent parent, JS9560 and donor parent, S14-9017GTforyield, yield component traits, when grown under herbicide sprayed conditions were recorded. All the data are presented in Table 3. While the donor plant recorded a height of 118 cm, the mean performance of BC_2F_3 for plant height was 121.07 cm, which was determined to be equivalent with the plant height (122 cm) expressed by the recurrent parent, JS 9560. Although the donor parent reported 31 days to flowering, the mean performance of BC_2F_3 plants for days to flowering was 27.34 days, which was comparable to the recurrent parent flowering period of 28 days. In contrast to the mean days for donor and recurrent parents, which were 95 and 82 days, respectively; the mean days to harvesting for BC_2F_3 was found to be 84 days.

In BC_2F_3 plants, the number of pods per plant and the number of seeds per plant were found to be 7 and 17, respectively, which were comparable to the number of pods per plant (8) and the number of seeds per plant (21) of the recurrent parent, JS 9560.The other important trait, 100 seed weight, on average, was recorded as 11.20 g in BC_2F_3, while it was 12.5g in the recurrent parent, JS 9560; however, the donor parent recorded 15.5g. The mean seed yield per plant in BC_2F_3 was recorded to be 1.91g which was almost similar with the seed yield (2.1g) of the recurrent parent, JS 9560, while the donor parent, S14-9017GT, recorded 2.8g of seed yield per plant.

Discussion

There are currently no soybean cultivars in India that are tolerant to the herbicide glyphosate. However, through the introduction of genes for proteins from *Agrobacterium* spp. strain CP4, the first genetically engineered soybean cultivars were created to be Tolerant to the herbicide glyphosate (Homrich et al. 2012). A gene encoding the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) has been introduced into soybeans that are tolerant to glyphosate. The American soybean variety S14-9017GT possesses the *EPSPS* gene, which provides glyphosate tolerance (Chen et al. 2020). In the present study, we adopted MABB approach using the S14-9017GT as donor parent to introgress the glyphosate tolerant gene into the genetic background of popular Indian soybean variety, JS 9560, and developed the first Indian glyphosate tolerant BC_2F_3 soybean lines.

Marker-assisted selection (MAS) is currently an effective technique for improving the precision and efficacy of soybean breeding (Wang et al. 2024). By utilising molecular markers linked to desired traits, MAS enables breeders to select plants early in the breeding process that have the suitable genetic makeup. The resources and time needed to generate new cultivars are significantly reduced by this strategy. Research has shown that MAS is useful in locating quantitative trait loci (QTLs) linked to key characteristics, which makes it easier to create soybean cultivars with desirable qualities. (Fields et al. 2023; Van and McHale 2017; Vargas et al. 2024). However, considering its important significance in enabling large-scale agriculture operations,

Table 3. Mean performance of morphological traits in BC_2F_3 Plants under glasshouse conditions

S. No.	Parental line	Plant height (cm)	Days to flowering	Days to harvesting	No. of pods per plant	No. of seeds per plant	100 seed weight g	Seed yield per plant g
1	JS 9560	122	28	82	8	21	12.5	2.1
2	BC_2F_3 plants mean	121.07	27	84	7	17	11.20	1.94
3	S14 9017GT	118	31	95	11	31	15.5	2.8

herbicide tolerance in soybeans continues to be the main target of genetic engineering for commercialisation goals (Vargas et al. 2024).

Transferring the desired glyphosate tolerance trait into the genetic background of JS 9560 using an American soybean variety is a difficult task, particularly when it comes to recovering the recurrent parent genome in the improved BC_2F_2 plants. The *EPSPS* allele for herbicide tolerance was precisely transferred as a result of foreground selection, background selection, and phenotypic selection. With just two backcross generations, the RPG recovery was expedited to 94.35 to 98.77%. With the highest RPG percentage of 98.77% in one BC_2F_2 plant (P-11-09), the *EPSPS* gene carrier chromosome and the rest of the chromosome exhibited full recovery of RPG (Table 2). Since SSR markers often target non-coding and heterochromatic regions, they are unable to quantify the recovery of the functional portion of the genome when used for background analysis. Because phenotypic selection for the recurrent parent phenome (RPP) involves indirect selection for the functionally expressed part of the genome, background selection was therefore combined with RPP to speed up the reconstruction process (Ellur et al. 2016a). The improved BC_2F_3 plants exhibited herbicide tolerance comparable to S14-9017GT and were comparable to JS 9560 in terms of yield and yield-contributing characteristics. In each of the backcross generations, the JS 9560 phenotype underwent rigorous phenotypic selection for yield and yield-related traits, thereby rendering this achievable. The significance of phenotypic selection with background selection in the development of back cross population with maximum RPG and RPP recovery has also been earlier demonstrated (Gaban, B.L. 2013; Pantalone et al. 2013; Kubo et al. 2013; Grover et al. 2020; Ellur et al. 2016b; Babu et al. 2017). Recently several studies have been conducted in the development of glyphosate-tolerant soybean cultivars using different modern plant breeding strategies. Mora-Oberlaender et al. (2024) developed glyphosate-tolerant soybean using an agbiogenetic model. It has been reported that the herbicide-tolerant traits in soybeans have been created by modification of different AHAS homologous alleles by base editor (Weiet al. 2023). G10-EPSPS, other amino acids from *Deinococcus radiodurans*, have been introduced into soybean, which conferred tolerance to glyphosate (Xiao et al. 2019). A superior BC_4F_2 glyphosate-tolerant soybean cultivar with drought-tolerant trait has also been created (Smallwood et al. 2024).

The primary desired characteristic in soybeans is herbicide tolerance, which is mainly controlled by the *CP4-EPSPS* gene (Padgette et al. 1995). Li et al. (2019) reported that plants with full glyphosate tolerance have successfully imported the CP4 5-enolpyhruvylshikimate-3-phosphate synthase (*CP4 EPSPS*) gene. Due to genetic alteration for 5-enolpyhruvylshikimate-3-phosphate synthase (*EPSPS*), glyphosate's target enzyme, glyphosate-tolerant soybeans survive glyphosate treatment. The shikimate pathway enzyme, *EPSPS*, is targeted by glyphosate. This enzyme is vital to plant life since it is required to produce phenylalanine, tryptophan, and tyrosine. When exposed to glyphosate, plants without the genetic modification die (Shinabarger and Braymer 1986; Xu et al. 2003). The CP4 5-enolpyhruvylshikimate-3-phosphate synthase (*CP4 EPSPS*) gene have been effectively imported by the 14 BC_2F_2 plants that were found to be homozygous for glyphosate tolerance following successful foreground selections in the current investigation.

In this study, it was recommended that glyphosate (3 ml/lit) be sprayed at the third trifoliate stage of soybeans, and the survival rates of the 38 BC_2F_2 soybean materials were counted subsequently. After two weeks of glyphosate treatment, about 14 BC_2F_2 were found to be tolerant (Table 3) and survived and were identified as complete homozygous (*EPSPS/EPSPS*) for the target gene. It indicated that the expression levels of the *EPSPS* gene in the backcrossed progeny were satisfactory. Earlier, Liu et al. (2024) reported that the expression of exogenous protein genes for glyphosate tolerant in backcrossed progeny is notably low. However, in the current study, we developed 583 glyphosate-tolerant BC_2F_3 plants and evaluated them for agronomic traits, specifically focusing on plant height (cm), number of pods per plant, number of seeds per plant, 100 seed weight, and seed yield per plant (Table 3). Overall, BC_2F_3 plants recorded almost similar comparable yield and yield-contributing traits with the recurrent parent, JS9560, indicating that the presence of the *EPSPS* gene did not significantly affect morphology.

To produce glyphosate-tolerant plants, a tolerant gene alone is not enough; the improved, tolerant cultivars must perform optimally for yield and yield-related traits to be developed commercially. In the present study, newly developed tolerant BC_2F_3 plants exhibited the best yield as well as yield-contributing qualities that were like recurring parent traits. This was made possible as the BC_2F_2 plants had the most recurrent parent genome recovery and enhanced BC_2F_3tolerant plants had rigorous phenotypic selection. Farmerscan benefit greatly from the creation of soybean cultivars that are more tolerant to both abiotic and biotic stresses, which reduces their reliance on chemical inputs like fertilisers and pesticides (Ben, R. et al. 2014). By incorporating traits such as drought tolerance, pest tolerance, and herbicide tolerance, soybean producers can significantly reduce production costs and environmental impacts, supporting sustainable agricultural practices (Fang et al. 2024; Qingfeng Niu et al. 2024; Wei et al. 2023).

Overall, in the current study, we successfully developed BC_2F_3 plants carrying the *EPSPS* allele that displayed tolerance to glyphosate herbicide when applied at 3 ml/litre with no

significant difference in the yield performance and other yield-related traits. This indicates the effectiveness of the EPSPS gene to overcome the adverse effect of the herbicide glyphosate. However, further evaluations are required for the commercial release of glyphosate-tolerant varieties, thus contributing to weed management in soybean production

Authors' contribution

Conceptualization of research (AT, OR); Designing of the experiments (AT,SV, L, RP, NB, RAR); Contribution of experimental materials (AT, MT, MY, MS); Execution of field/ lab experiments and data collection (OR, MS, NKKR, MT, MY, SBR, KJ); Analysis of data and interpretation (OR, AT, NB); Preparation of the manuscript (OR, AT, MS, SKL).

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