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Evaluation of the usefulness of senescing agent potassium iodide (KI) as a screening tool for tolerance to terminal drought in soybean

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Abstract

Drought at seed fill stage is a major limitation to soybean productivity in countries where crop is mainly grown on seasonal rains. Improved translocation of stem reserves to developing seeds under such a condition could play an important role in improving the productivity of soybean. However, uncertain nature of rains makes it difficult to evaluate large number of genotypes for this trait under field conditions. Chemical desiccants such as potassium iodide (KI) have been successfully used to evaluate genotypes for terminal drought in many crops, but their use in soybean has not been reported. In this study, efforts were made to evaluate the usefulness of KI in identification of soybean genotypes tolerant to terminal drought. Three concentrations of KI (0.1, 0.2 and 0.3 %) were sprayed on soybean variety JS 335 at the beginning of seed fill (R5 stage) and, 7, 14, 21 and 28 days after R5 stage for two years (2008 and 2009). Based on relative losses in seed yield and 100 seed weight as compared to untreated control, it was observed that spraying of 0.1 to 0.2 % KI between 7 to 14 days after R5 stage can simulate terminal drought appropriately. Subsequently, 200 soybean genotypes were evaluated under field condition for relative tolerance to terminal drought by spraying 0.2 % KI at 10 days after plants reached to R5 stage. Three genotypes (EC 538828, JS 97-52 and EC 602288) were identified as showing relative tolerance to terminal drought as compared to other genotypes. These genotypes were further tested under natural drought at seed fill stage in rainout shelters. The reduction in seed yield (20-31 %) was relatively less in the identified genotypes as compared to other genotypes (34-60 %). It is concluded that KI can be used as chemical desiccant for field evaluation of large number of soybean genotypes and identification of genetic sources for tolerance to terminal drought.

Key words: Chemical desiccant; Potassium iodide; Soybean; Seed size; Seed yield; Terminal drought.
Abbreviations: KI_ potassium iodide; DAC_ Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India; R3 stage_ beginning pod stage, R5 stage_ beginning seed stage in soybean; m_ meter; CV_ Coefficient of variation.

Introduction

Soybean [*Glycine max* (L.) Merrill] is the most important grain legume of the world, which contributes significantly to edible oil, protein concentrate for animal feed, food uses and various industrial products. Since 1970, the average annual increase in soybean area has been the highest among the major crops of the world (Hartman et al., 2011). India has been one of the major contributors to global soybean expansion during the past 40 years. Starting with an area and production of just 30, 000 ha and 14,000 tons in 1970, the crop has expanded to 10.5 million ha with 12.4 million tons of production in 2012 (DAC, 2013). Currently soybean, a leading oilseed crop in India, is grown by millions of small and marginal farmers in rainfed agro-ecosystem. However, its average productivity has remained more or less stagnated at 1-1.2 t ha^{-1}, which is less than half of the world average and one third of its climatic potential (Bhatia et al., 2008).
Globally, drought is one of the major factors responsible for reducing the soybean productivity below its climatic potential. Annually, about 40 % reduction in soybean yield due to drought has been reported (Specht et al., 1999). However, depending upon the intensity and the stage at which drought occurs, the losses could be as high as 80 % (Oya et al., 2004; Dias et al., 2012). In India also, drought is the most important factor determining the soybean productivity owing to total dependence of its cultivation on seasonal (monsoon) rains. These monsoon rains often show wide spatial and temporal variations (Joshi and Bhatia, 2003) resulting in dry spells of varying duration at one or the other stage(s) of crop growth. However, the frequency of occurrence of drought at terminal phase of soybean (seed filling and, after pod and seed numbers are fixed) due to early cessation of monsoon rains is the maximum. Drought during vegetative phase is compensated to some extent by the subsequent rainfall while the stress during seed fill stage has often no chance of recovery (Sionit and Kramer, 1977; Hirasawa et al., 1994; Saitoh et al., 1999) and hence, results in severe loss of soybean productivity. Terminal drought leads to reduced photosynthesis, rapid senescence of leaves, forced maturity and severe reduction in yield due to reduced seed filling (Brevedan and Egli, 2003; Manavalan et al., 2009). The reduced photosynthesis and rapid senescence of leaves obstruct the supply of assimilates available to seed ultimately affecting the seed size in soybean. Under such a situation, remobilization of reserves stored in stem become an important contributor to the requirements of seed filling and seed yield in legumes including soybean (Constable and Hearn, 1978). These carbohydrate reserves act as a buffer against availability of current photosynthates particularly during seed filling (Schnyder, 1993; Subbarao et al., 1995). In soybean, about 25 % of the seed weight is reported to be produced from stem reserves (Constable and Hearn, 1978). Hence, the ability to store and mobilize higher quantities of

stored reserves for seed filling under terminal drought should improve the ability of a cultivar to perform better. Field evaluation of large germplasm and identification of genetic resource having larger contribution of reserves and hence relative tolerance to terminal drought is difficult due to uncertain nature of rains. Also, year to year variations in soil moisture deficit makes reliable selection for the character difficult in dry land breeding programmes (Regan et al., 1993). A frequently employed screening technique is to inhibit photosynthetic capacity at seed filling stage by spraying plants with potassium iodide (KI) solution (Blum et al., 1983ab, Regan et al., 1993). KI acts as a desiccant and inhibits photosynthesis without a detrimental effect on translocation of assimilates to developing grains. The response of plants to potassium iodide is primarily due to desiccation and it mimics the terminal drought stress on physiological and biochemical parameters, such as reduction in rate of photosynthesis and chlorophyll content, senescence, increase in sucrose and proline content (Sawhney and Singh, 2002). This technique has been extensively employed for large scale field evaluation of genotypes for tolerance to terminal drought in many cereal crops (Nicolas and Turner, 1993; Royo and Blanco, 1998, Ashraf et al., 2003; Singh et al., 2012) and the reduction in grain dry weight has been found to be comparable with that obtained under natural drought conditions. However, there are limited efforts to use this technique to identify the genotypes tolerant to terminal drought in legumes including soybean. Considering the importance of terminal drought and significant role of remobilization of stem reserves to grain filling under drought conditions, an attempt was made to use this technique for evaluation of soybean genotypes for tolerance to terminal drought. The main objectives of the study were to i) evaluate the efficacy of KI as a tool to screen soybean genotypes for terminal drought tolerance, ii) standardize the KI technique in terms of concentration and time of application for field evaluation of soybean genotypes, iii) evaluate soybean genotypes for relative tolerance to terminal drought using KI and iv) confirm the relative tolerance in identified genotypes under natural drought conditions.

Results and discussion

Standardization of concentration and time of application of KI

Depending on climatic conditions, the average soybean yield and 100 seed weight were significantly higher in 2009 (2321 kg ha^{-1} and 10.2 g) as compared to 2008 (1892 kg ha^{-1} and 9.5 g) (Table 1 and 2). The higher productivity levels during 2009 could be associated with better solar radiation particularly during reproductive phase as compared to 2008 (Fig 1). The reduced solar radiation due to heavy cloudy conditions during rainy season is an important factor responsible for yield variations across years and locations (Bhatia et al., 2008). Relatively higher temperatures during 2009 crop season resulted in reducing maturity period by three days (88 days) as compared to 2008 (91 days) (Table 3).

The average seed yield in untreated control plots during 2008 and 2009 was 2510 and 2980 kg ha^{-1} with an average value of 2745 kg ha^{-1} (Table 1). The seed yield was significantly reduced when 0.1, 0.2 and 0.3 % KI was sprayed on the plants at different stages of seed development. On an average, the reduction was 24 and 28 % when 0.1 and 0.2 % KI was sprayed while it was very high (42 %) in plants sprayed with 0.3 % KI. Among the three concentrations of KI, the seed yield of plants sprayed with 0.3 % was significantly lower during 2008 and 2009 (1396 and 1799 kg ha^{-1}) as compared to plants treated with 0.1 % (1888 and 2301 kg ha^{-1}) and 0.2 % (1775 and 2207 kg ha^{-1}), respectively. However, the difference between the seed yield of plants treated with 0.1 and 0.2% KI was non-significant. The reduction in seed yield was mainly associated with reduction in seed size as the trend in reduction in 100 seed weight was similar to seed yield (Table 2). The average 100 seed weight of untreated control plants was 12.5 g and was on an average reduced by 19, 26 and 38 % in the plants treated with 0.1, 0.2 and 0.3 % KI, respectively. The duration of the crop was moderately but significantly reduced in both the years when 0.1, (90 days), 0.2 (88 days) and 0.3 % (87 days) of KI was sprayed as compared to untreated control (94 days) (Table 3). It is important to identify the time of application of chemical desiccant for its effective use in simulating terminal drought (Turner et al., 1989). In this study, a severe reduction in seed yield was observed (43%) in soybean plants when KI was sprayed immediately after the plants reached to beginning seed stage (R5 stage) as compared to the plants where spray was made 28 days after R5 stage (Table 1). The magnitude of yield loss declined as the spray of KI was delayed after seed filling began and the yield loss was negligible when the spray was made 28 days after plants reached to R5 stage (2701 kg ha^{-1}) as compared to untreated control. The average 100 seed weight reduced in the same manner as that of seed yield when KI was sprayed at different stages after seed filling began (Table 2). The reduction in crop duration was relatively higher when KI was sprayed at early stages of seed filling as compared sprays made at later stages (Table 3). The interactions of year x KI concentration and year x time of KI application were not significant while that of KI concentration x time of KI application was highly significant. Therefore, the pooled data was subjected to factorial analysis and is presented in Table 4. The significant interaction between KI concentration and time of application indicated that reduction in seed yield and 100 seed weight varied widely in response to KI concentrations sprayed at different stage of seed formation in soybean. Compared to untreated control plants, significant reduction in seed yield was observed when 0.1, 0.2 and 0.3 % KI was sprayed at R5 stage and, 7, 14 and 21 days after R5 stage (Table 4). Spray of KI did not reduce the seed yield significantly when sprayed 28 days after R5 stage. However, the magnitude of reduction in seed yield was of greater proportion when 0.3 % KI was sprayed at these stages as compared to spray of 0.2 and 0.1 % (Fig 2a). Compared to untreated control, the reduction in seed yield in plants sprayed with 0.1, 0.2 and 0.3 % KI at R5 stage was 48, 53 and 76 %, respectively. Similar reduction in yield when KI was sprayed at 7 days was 39, 45 and 65 % and at 14 days after R5 stage was 24, 30 and 54 %, respectively. The reduction in yield as compared to untreated control plants when same concentrations of KI were sprayed at 21and 28 days after R5 stage was very low and ranged from 6-10 and 1-4 %, respectively. The seed filling is adversely affected due

Table 1. Effect of different concentrations and time of application of chemical desiccant (KI) on seed yield of soybean (Pooled data for 2008 and 2009).

Treatments	Seed yield (kg ha^{-1})			Reduction in yield (%)
	2008	2009	Pooled	
Concentration of KI				
Untreated control	2510^{a}	2980^{a}	2745^{a}	-
0.1%	1888^{b}	2301^{b}	2095^{b}	23.7
0.2%	1775^{b}	2207^{b}	1991^{c}	27.5
0.3%	1396^{c}	1799^{c}	1598^{d}	41.8
LSD ($p \leq 0.05$)	124.8	124.6	86.9	-
Time of application of KI				
R5 stage	1382^{c}	1683^{e}	1533^{e}	43.2
R5 + 7 days	1490^{c}	1948^{d}	1719^{d}	36.4
R5 + 14 days	1780^{b}	2232^{c}	2006^{c}	25.7
R5 + 21 days	2356^{a}	2795^{b}	2576^{b}	4.6
R5 + 28 days	2453^{a}	2948^{a}	2701^{a}	-
LSD ($p \leq 0.05$)	139.6	139.3	97.1	-
Pooled analysis				
Year	1892^{b}	2321^{a}	2107	-
LSD ($p \leq 0.05$)			61.4	

Means within a column for each main treatment followed by the same letters are not significantly different ($p \leq 0.05$).

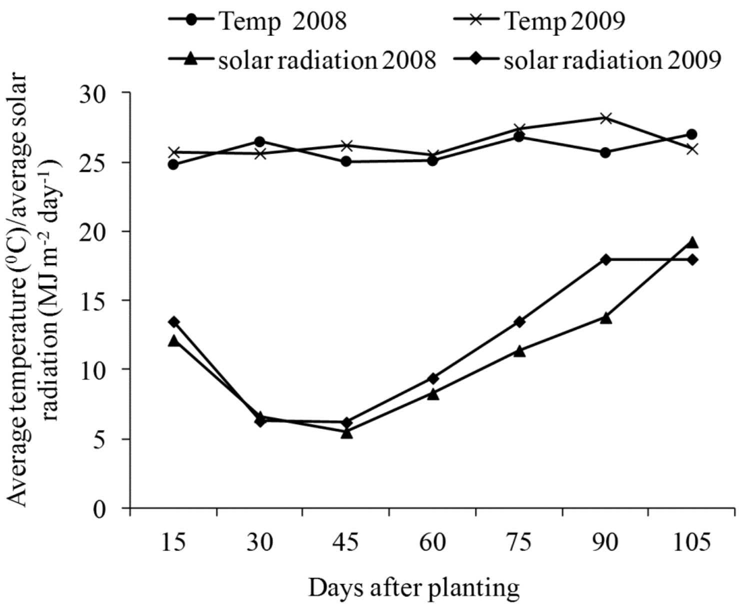

Fig 1. Average temperatures and solar radiation during the crop growth period (2008 and 2009)

to non-availability of photosynthates, mainly caused by reduced rate of photosynthesis and senescence of leaves (Brevedan and Egli, 2003; Manavalan et al., 2009). Spray of chemical desiccants mimics the similar conditions by reducing the photosynthesis and rapid senescence of leaves. The reduction in seed yield in the desiccated plants has been shown to be closely correlated with the reduction in seed weight in other crops (Nicolas and Turner, 1993). In the present study, desiccation of soybean plants with KI resulted in reduced seed weight (Table 4). The reduction in seed weight followed the similar trend as that of reduction in seed yield (Fig 2b). The 100 seed weight of untreated control plants (12.5 g) was significantly higher than the plants treated with three concentrations of KI sprayed at R5 stage and at 7, 14 and 21 days after R5 stage. Among the three concentration, 100 seed weight in plants sprayed with 0.3 % KI at R5 stage (3.9 g), 7 (5.7 g) and 14 days after R5 stage (6.7 g) was significantly lower than plants sprayed with 0.1 and 0.2 % KI. Compared to untreated control, the reduction in 100 seed weight in plants treated with 0.1, 0.2 and 0.3 % KI at R5 and, 7 and 14 days after R5 stage was 40, 46 and 69 %; 26, 41 and 54% and, 21, 32 and 46 %, respectively (Fig 2b). The results thus indicated that the effect of 0.3 % KI concentration was very severe on seed yield and seed weight, while effect of 0.1 and 0.2 % were moderate. Also, the effect of KI was very high when sprayed immediately after R5 and 7 days after R5 stage, while it became moderate when KI was sprayed at 14 days after the crop reached to R5 stage. The

Table 2. Effect of different concentrations and time of application of chemical desiccant (KI) on 100 seed weight of soybean (Pooled data for 2008 and 2009).

Treatments	100 seed weight (g)			Reduction in 100 seed weight (%)
	2008	2009	Pooled	
Concentration of KI				
Untreated control	12.2^{a}	12.8^{a}	12.5^{a}	-
0.1%	9.6^{b}	10.4^{b}	10.1^{b}	19.2
0.2%	8.7^{bc}	9.5^{b}	9.2^{c}	26.4
0.3%	7.3^{c}	8.2^{c}	7.8^{d}	37.6
LSD ($p \leq 0.05$)	1.23	1.06	0.80	-
Time of application of KI				
R5 stage	6.9^{c}	8.1^{c}	7.7^{c}	38.4
R5 + 7 days	8.0^{b}	9.5^{b}	8.7^{b}	30.4
R5 + 14 days	9.3^{b}	9.7^{b}	9.4^{b}	24.8
R5 + 21 days	11.2^{a}	11.6^{a}	11.4^{a}	8.8
R5 + 28 days	12.0^{a}	12.2^{a}	12.2^{a}	-
LSD ($p \leq 0.05$)	1.37	1.18	0.89	-
Pooled analysis				
Year	9.5^{b}	10.2^{a}	9.9	-
LSD ($p \leq 0.05$)			0.56	-

Means within a column for each main treatment followed by the same letters are not significantly different ($p \leq 0.05$).

loss in seed yield under natural terminal drought varies depending on the severity of the stress, nonetheless on an average 30-40 % yield losses have been reported in soybean (Specht et al., 1999). Therefore, based on the yield losses and reduction in seed size, it was concluded that the spray of KI between 0.1 and 0.2 % between 7-14 days after the crop reached R5 stage could mimic the terminal drought appropriately.

Evaluation of soybean germplasm for tolerance to terminal drought using KI

In order to identify genetic resources for relative tolerance to terminal drought, 200 soybean genotypes were evaluated using KI. The average seed yield of these genotypes in untreated control plants was 1612 kg ha^{-1}, which ranged from 568 to 2793 kg ha^{-1} (Table 5). When 0.2 % KI was sprayed at 10 days after the plants reached to R5 stage, the average seed yield of these genotypes was 674 kg ha^{-1} with a range of 218 to 2083 kg ha^{-1}. Hence, an average reduction of 59 % in seed yield was observed when KI was sprayed on these lines as compared to untreated control plants. Among genotypes evaluated, the reduction in yield ranged from 18 to as high as 71 %. On per plant basis also, the average reduction in seed yield in plants treated with KI was 56 % with a range of 21 to 79 % as compared to untreated control plants. Average 100 seed weight of untreated control plants of these genotypes was 11.1 g which was reduced to 5.7 g when plants were sprayed with KI. The average reduction in100 seed weight was 49 % and magnitude of reduction ranged from 17 to 67 %. The results thus indicated that there was a large genotypic variability in response to terminal drought. The average days to maturity was 100 days for untreated control plants as compared 96 days in plants treated with KI. Also the effect of KI on maturity was not consistent as the maturity in some genotypes was slightly earlier and delayed in others as compared to untreated control plants. Among 200 soybean genotypes evaluated, three were identified as possessing

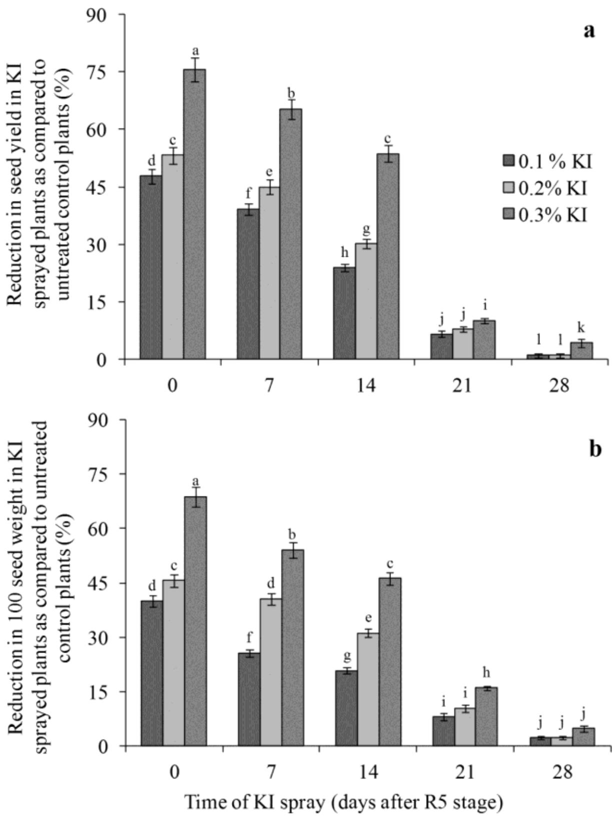

Fig 2. Reduction in (a) seed yield and (b) 100 seed weight in soybean when different concentrations of KI were sprayed at different stages of seed fill (pooled data for 2008 and 2009). Bars above each column indicate standard error of mean (±) and same letters above columns indicate that the means are not significantly different ($p \leq 0.05$).

Table 3. Effect of different concentrations and time of application of chemical desiccant (KI) on days to maturity of soybean (Pooled data for 2008 and 2009).

Treatments	Days to maturity			Reduction in maturity (%)
	2008	2009	Pooled	
Concentration of KI				
Untreated control	95^{a}	92^{a}	94^{a}	-
0.1%	91^{b}	88^{b}	90^{b}	4.2
0.2%	90^{b}	87^{b}	88^{c}	6.4
0.3%	90^{b}	85^{c}	87^{c}	7.4
LSD ($p \leq 0.05$)	1.3	1.4	1.0	-
Time of application of KI				
R5 stage	90^{c}	87^{d}	88^{b}	6.4
R5 + 7 days	90^{c}	86^{cd}	88^{b}	6.4
R5 + 14 days	91^{bc}	88^{bc}	89^{b}	5.3
R5 + 21 days	92^{ab}	89^{b}	91^{a}	3.2
R5 + 28 days	93^{a}	91^{a}	92^{a}	2.1
LSD ($p \leq 0.05$)	1.4	1.6	1.1	-
Pooled analysis				
Year	91^{a}	88^{b}	90	
LSD ($p \leq 0.05$)			0.67	

Means within a column for each main treatment followed by the same letters are not significantly different ($p \leq 0.05$).

Table 4. Interactive effect of concentrations and time of application of chemical desiccant (KI) on Seed yield and 100 seed weight of soybean (Pooled data for 2008 and 2009).

Concentration	Seed yield (kg ha^{-1})					100 seed weight (g)				
	Time of application (Days after R5 stage)					Time of application(Days after R5 stage)				
	0	7	14	21	28	0	7	14	21	28
Untreated control	2745^{a}	2745^{a}	2745^{a}	2745^{a}	2745^{a}	12.5^{a}	12.5^{a}	12.5^{a}	12.5^{a}	12.5^{a}
0.1%	1432^{f}	1670^{e}	209^{d}	2564^{abc}	2716^{ab}	7.5^{fg}	9.3^{de}	9.9^{cde}	11.5^{abc}	12.2^{ab}
0.2%	1283^{g}	1510^{ef}	1918^{d}	2527^{bc}	2717^{ab}	6.8^{gh}	7.4^{fgh}	8.6^{ef}	11.2^{abc}	12.2^{ab}
0.3%	670^{i}	952^{h}	1271^{g}	2468^{c}	2628^{abc}	3.9^{i}	5.7^{h}	6.7^{gh}	10.5^{bcd}	11.9^{ab}
LSD ($p \leq 0.05$)										
Concentration x time of application			194.3			1.78				

Means followed by the same letters are not significantly different ($p \leq 0.05$).

Table 5. Seed yield, 100 seed weight and seeds pod^{-1} of 200 soybean genotypes evaluated for terminal drought using chemical desiccant (KI).

Characters	Untreated control			Chemical desiccation			Percent reduction due to chemical desiccation		
	Mean	Range	CV (%)	Mean	Range	CV (%)	Mean	Range	CV (%)
Seed yield (kg ha^{-1})	1612	568-2793	29.0	674	218-2083	48.1	58.5	18-71	19.9
Seed yield (g pl^{-1})	4.9	1.61-9.2	30.8	1.8	0.66-6.0	37.3	56.2	21-79	14.9
100 Seed weight (g)	11.1	5.3-24.2	25.3	5.7	2.4-20.0	35.5	48.5	17-67	18.4
Seeds pod^{-1}	1.5	0.67-2.7	25.2	1.3	0.6-2.5	26.4	10.2	0.3-21	55.8
Days to maturity	99.6	71-123	8.6	96.0	75-118	8.2	4.0	-6 - 4	93.4

relative tolerance to terminal drought based on the minimum reduction (less than 30 %) in seed yield and 100 seed weight in plants treated with KI as compared to untreated control plants (Table 6). These genotypes included two exotic germplasm accessions (EC 538828 and EC 602288) and one released soybean cultivar JS 97-52. The seed yield of untreated control plants of EC 538828, JS 97-52 and EC 602288 was 2498, 2711 and 1940 kg ha^{-1}, which was reduced by 18, 23 and 25 % in plants sprayed with KI, respectively. Similarly, the 100 seed weight of untreated control plants of EC 538828, JS 9752 and EC 602228 was 24.2, 8.9 and 8.4 g, which was reduced to 20.0, 6.9 and 6.5 g in plants sprayed with KI, respectively. Hence, compared to untreated control plants, the reduction in seed weight was 17 % for EC 538828 and 23 % for JS 9752 and EC 602288. Genotype EC 538828 is determinate type with large seed size and matures early (86 days) as compared to JS 97-52 and EC 602288, which are semi-determinate, small seeded and late maturing (102 and 103 days). Among 200 soybean genotypes evaluated, the

magnitude of reduction in seed yield and seed weight was the lowest for EC 538828 followed by JS 97-52 and EC 602288 and hence were considered to have relative tolerance to terminal drought. The relative tolerance could be associated with greater seed filling due to more partitioning of photosynthetic reserves to developing seeds after the stress was imposed as has been reported in other crops (Nicolas and Turner, 1993; Ashraf et al., 2003; Singh et al., 2012). In case of soybean, the rate of seed filling has also been reported to be associated with high yield (Guffy et al., 1991). It is possible that faster rate of seed development could also have played a role in relative tolerance to terminal drought as by the time KI was sprayed (10 days after R5 stage) the filling of seeds in these genotypes could have been higher than other genotypes.

Table 6. Lines identified for tolerance to terminal drought using chemical desiccant (KI).

Characters	EC 538828	JS 9752	EC 602288
Seed yield (kg/ha)			
Untreated control	2498	2711	1940
Chemical desiccant	2036	2083	1450
Reduction due to chemical desiccant (%)	18.4	23.2	25.3
100 seed weight (g)			
Untreated control	24.2	8.9	8.4
Chemical desiccant	20.0	6.9	6.5
Reduction due to chemical desiccant (%)	17.3	22.5	22.6
Days to maturity			
Untreated control	86	102	103
Chemical desiccant	83	95	95
Reduction due to chemical desiccant (%)	3.5	6.9	7.8
Origin	USA	India	Brazil

Table 7. Comparison of seed yield of identified soybean genotypes with genotypes of similar maturity duration grown under irrigated and soil moisture stress at seed fill stage.

Genotypes (G)	Seed yield (kg ha^{-1})			
	Soil Moisture treatment (SM)			
	Irrigated	Stress at Seed fill	Mean	Yield reduction (%)
Early maturing				
EC 538828	2058^{a}	1654^{b}	1856^{A}	20
JS 95-60	2283^{a}	1400^{c}	1841^{A}	39
Samrat	2146^{a}	852^{d}	1499^{B}	60
NRC 7	2231^{a}	1459^{bc}	1845^{A}	35
MACS 330	1681^{b}	897^{d}	1289^{C}	47
NRC 12	1661^{b}	1094^{d}	1378^{BC}	34
Mean	2010$^{\alpha}$	1226$^{\beta}$	1618	39
LSD ($p \leq 0.05$)				
Soil moisture		264.4		
Genotypes		178.1		
SM x G		251.9		
Late Maturing				
JS 9752	2256^{ab}	1600^{c}	1928^{A}	29
EC 602288	2096^{b}	1453^{c}	1774^{AB}	31
NRC 37	2437^{a}	924^{d}	1681^{BC}	62
Hardee	2297^{ab}	1515^{c}	1906^{A}	34
NRC 2	2036^{b}	1022^{d}	1529^{CD}	50
Punjab 1	1710^{c}	1051^{d}	1381^{D}	39
Mean	2139$^{\alpha}$	1261$^{\beta}$	1700	41
LSD ($p \leq 0.05$)				
Soil moisture		250.3		
Genotypes		213.4		
SM x G		301.9		

Treatment means followed by the same letters (genotypes means with bold, soil moisture with Greek and interaction with small letters) are not significantly different ($p \leq 0.05$).

Yield of identified genotypes under natural terminal drought

In order to confirm the relative tolerance for terminal drought in the identified soybean genotypes, these were tested under natural drought conditions along with prominent varieties/genotypes. EC 538828 which flowered in 32 days and matured in 86 days was tested with five varieties/genotypes having similar days to flowering and days to maturity. Genotypes JS 97-52 and EC 602288 flowered in 46 days and matured in 102 days were compared with four varieties with similar days to flowering and days to maturity. The average yield of early and late soybean genotypes was 2010 and 2139 kg ha^{-1}, which was significantly reduced to 1226 and 1261 kg ha^{-1} under terminal drought conditions (Table 7). The average reduction in yield due to drought was 39 and 41 % for early and late maturing genotypes. Among early genotypes, the seed yield of EC 538828 under irrigated conditions (2058 kg ha^{-1}) was on par with JS 95-60, (2283 kg ha^{-1}), NRC 7 (2231 kg ha^{-1}) and Samrat (2146 kg ha^{-1}), while it was significantly higher as compared to MACS 330 (1681 kg ha^{-1}) and NRC 12 (1661 kg ha^{-1}). Under drought conditions, the highest yield was observed in EC 538828 (1654 kg ha^{-1}), which was significantly higher as compared to rest of the varieties except for NRC 7 (1459 kg ha^{-1}). The magnitude of reduction in seed yield of plants subjected to drought conditions as compared to irrigated plants was lowest for the genotype EC 538828 (20 %), which was identified as tolerant to terminal drought using KI spray. In rest of the genotype the magnitude of reduction ranged from 34 to 60 %. Under irrigated conditions, the highest seed yield among late maturing genotypes was observed for NRC 37 (2437 kg ha^{-1}) and was at par with the yields observed in genotypes Hardee (2297 kg ha^{-1}) and JS 97-52 (2256 kg ha^{-1}).

In plants subjected to terminal drought, the seed yield was highest for JS 97-52 (1600 kg ha^{-1}) and was at par with yields observed in genotypes Hardee (1515 kg ha^{-1}) and EC 602288 (1453 kg ha^{-1}). As compared to irrigated plants, the magnitude of reduction in seed yield under drought conditions ranged from 29 to 62 %. The percent reduction in genotypes JS 97-52 and EC 602288 identified as tolerant to terminal drought through KI was low 29 and 31 % as compared to rest of the genotypes. Among the genotypes tested, NRC 7 and Hardee were reported earlier also as relatively drought tolerant based on field evaluation under irrigated and rainfed conditions (Joshi and Bhatia, 2003). In the present study, lines identified as tolerant to terminal drought using KI spray (EC 538828, JS 97-52 and EC 602288) showed less reduction in seed yield as well as seed weight compared to rest of the varieties tested. This indicated that lines identified using KI do possess relative tolerance to terminal drought, which could be associated with higher translocation of stem reserves to developing seeds and hence, KI can be used for large scale screening of soybean genotypes possessing tolerance to terminal drought.

Materials and Methods

Standardization of concentration and time of application of KI

Before a chemical desiccant is used in a crop for field evaluation of genotypes for terminal drought, it is necessary to standardize the concentration and time of its application (Regan et al., 1993). Therefore, field trials were conducted during rainy season (June-October) of 2008 and 2009 at National Research Centre for Soybean, Indore (22.78 ^{0}N, 75.88 ^{0}E), India. The experimental site has black (Vertisols) soils with high to moderate depth, high water holding capacity and medium fertility. Soybean variety JS 335, which is the most popular and predominant variety in India (Bhatia et al., 2008) was planted in a randomized block design with three replications. Each plot consisted of 6 rows of 6 m length spaced at 0.45 m. Before planting, seeds were treated with recommended fungicide and inoculated with Bradyrhizobium culture. Recommended dose of fertilizers (NPK @ 20:26:17 kg ha^{-1}) was applied at the time of planting. Plants were kept free from soil moisture stress by applying irrigation as and when required. Standard agronomic practices for weed and insect control were uniformly followed. In addition to untreated control, the treatments comprised of three concentrations of KI (0.1, 0.2 and 0.3%) each sprayed uniformly at five seed fill stages viz. R5 stage (beginning seed fill) (Fehr et al., 1971) and, 7, 14, 21 and 28 days after R5 stage. Aqueous solution of KI was sprayed uniformly on the whole plant canopy to full wetting. Care was taken to protect other plots by putting a polythene screen around the plot sprayed with KI. At maturity, yield and its attributes were recorded. The per cent loss in yield and other attributes in KI sprayed plants was calculated (Regan et al., 1993) as: % reduction=100 *(C-S)/C, where C and S are the values of untreated control and stress treatment through KI spray, respectively.

Evaluation and identification of soybean genotypes for tolerance to terminal drought

Once the KI technique was standardized in terms of concentration of KI and time of its application, 200 diverse soybean genotypes that included soybean cultivars and, indigenous and exotic germplasm collections were evaluated during 2010 and 2011 for relative tolerance to terminal drought conditions. Two sets of 100 soybean genotypes (supplementary data) were planted under irrigated conditions in each year in a single row of 6 m length paced at 0.45 m. One set was kept as unsprayed control and in other set, each genotype was sprayed with 0.2% KI at R5+10 days stage. Both the KI treated and untreated sets were irrigated whenever required and similar agronomic practices as described above were followed. At maturity, 10 plants of each genotype were harvested and data on seed yield and yield attributes were recorded. Seed yield of rest of the plants in 6 meter row were also recorded.

Field evaluation of identified genotypes

Three genotypes that included one released cultivar JS 97-52 and two exotic germplasm lines EC 538828 and EC 602288 identified as possessing relative tolerance to drought were field evaluated for natural terminal drought during rainy season of 2012 along with popular soybean varieties. Among the identified lines, two (JS 97-52 and EC 602288) were late maturing types and one (EC 538828) was early maturing type. Therefore, 6 early and 6 late maturity genotypes including the identified genotypes were planted in a split plot design with three replications. The main plots consisted of irrigated plots and plots subjected to natural drought at reproductive stage under rainout shelter conditions, while subplots constituted 6 genotypes each in early and late maturing soybean genotypes. Two rainout shelters were used one each for early and late maturity genotypes. The plants till R3 stage (pod initiation) (Fehr et al., 1971) were kept open and were irrigated whenever required. After R3 stage rainout shelters were activated so that during rains, plants were protected from water. The severe drought conditions under rainout shelter occurred 10-12 days after the plants reached to R5 stage. The data on seed yield and its attributed was recorded at maturity and was subjected to analysis of variance using irrigation treatment as main plots and genotypes as subplots.

Statistical analysis

The experiment related to standardization of concentration and time of application of KI was carried out in a randomized block design with three replications for two years (2008 and 2009). Analysis of variance using two factors (concentration and time of application of KI) for each year (2008 and 2009) as well as for pooled data was performed. The evaluation of 200 soybean genotypes was carried out using augmented design and the mean, range and coefficient of variation (CV) for each character were calculated. Field evaluation of identified genotypes along with popular varieties was carried out in a split design with three replications. The analysis of variance was carried out using MSTATC software and the treatment means were compared based on least significant differences (LSD) at $p<0.05$.

Conclusions

Terminal drought is a serious problem to soybean production in countries such as India where the crop is grown mainly on seasonal rains. It occurs at such a stage of the crop wherein among other traits, the increased mobilization of stored reserves under drought conditions could contribute to increased tolerance to terminal drought. A technique involving spray of potassium iodide under irrigated condition has successfully been used in number of cereal and other crops for field evaluation of genotypes having increased contribution of stem reserves to developing grains and hence tolerance to terminal drought. As there are hardly any study indicating the use of this technique in legume crops, attempts were made in this study to find out its potential use in soybean. The results of the current study indicated that spray of 0.1 to 0.2 % KI between 7-14 days after the crop reaches the R5 stage can mimic the terminal drought in soybean and could help in field evaluation of large number of genotypes having relative tolerance to terminal drought. Using KI technique, 200 soybean genotypes were evaluated and three lines (EC 538828, JS 97-52 and EC 692288) were identified, which showed significantly lower reduction in seed yield and seed weight. The identified lines also performed relatively better under natural drought created at seed fill stage under rainout shelters. These lines are potential genetic sources for breeding soybean for tolerance to terminal drought conditions. It is concluded that KI can be used as a potential tool to field evaluate large number of soybean genotypes possessing relative tolerance to terminal drought in soybean.

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