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Field Crops Research 36 (1994) 103–111

Field Crops Research

Use of the senescing agent potassium iodide to simulate water deficit during flowering and grainfilling in pearl millet

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(Received 25 January 1993; accepted 24 November 1993)

Abstract

Grain growth in cereal crops is largely dependent on the availability of current photosynthates. Water deficits during flowering and grainfilling limit grain yield partly by reducing the availability of current assimilates. Drought response of breeders lines is often evaluated by screening material in drought prone environments or during the rain-free season by withholding irrigation during the desired treatment periods. The former approach is time consuming due to the erratic nature of drought patterns. In the latter approach, temperatures and daylength during the dry periods often affect crop growth and limits its use. It was therefore hypothesized that the plant photosynthetic source could be progressively destroyed in a controlled manner using a chemical desiccant or senescing agent to simulate water deficit effects. The effects of spraying the senescing agent potassium iodide (KI), at different stages before and after anthesis, on grain yield and yield components were compared with water deficit during grainfilling using four millet hybrids grown under field conditions in 1989 and 1991.

The senescing agent KI reduced the chlorophyll content of the leaves more severely than did the water deficit treatment. Spraying with KI reduced grain yield, grain number and grain size. Effects of KI spraying at anthesis on grain yield and yield components were similar in both years and were similar to the effects of water deficits during grainfilling in 1989. Spraying with KI at the anthesis stage for each hybrid eliminated the confounding effects of phenology often encountered in water deficit treatment. We conclude that in pearl millet, spraying a senescing agent at anthesis is effective in simulating the reduction of current photosynthesis that occurs during posts anthesis water deficit and can be used as a screening method to evaluate genetic response to water deficit during grainfilling in the normal season.

Key words: Chlorophyll content; Pearl millet; *Pennisetum*; Senescing agent; Water deficit

1. Introduction

Severe post-anthesis water deficits limiting grain production are characteristic of the environments in Africa and South Asia where pearl millet [*Pennisetum glaucum* (L.) R.Br.] is grown. Under these conditions, improved drought adaptation to post-anthesis drought stress is an important objective of the plant breeding programs for this crop. Traditionally, the drought response of breeding lines is evaluated by screening material in natural stress environments. This approach is time consuming due to the erratic nature of drought patterns in these environments. Alternatively, drought responses of rainfed crops have been evaluated by growing the crop during rain-free periods with irriga-

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SSDI 0378-4290(93)E0069-A

tion and imposing drought treatment by withholding irrigation during the desired treatment periods. Low temperatures during early growth, high temperatures during grainfilling and short daylengths often result in poor early growth, earlier flowering and reduced grain size, which limits the use of such techniques. In pearl millet, ability to set and fill grains under water deficit conditions was found to vary among cultivars (Bidinger et al., 1987). Further, genetic variation for this trait was related to flowering time of cultivars in relation to onset of stress (Fischer and Maurer, 1978; Bidinger et al., 1987; Mahalakshmi et al., 1988).

Treatment of wheat plants with a chemical desiccant to arrest current photosynthesis at 10 to 14 days after anthesis was found to simulate grain growth under post-anthesis drought stress (Blum et al., 1983a,b). Turner et al. (1989) compared a range of chemical desiccants and senescing agents and found potassium iodide sprayed after anthesis more closely simulated effects of drought than did other chemicals. Further, KI treatment consistently ranked varieties for grain size stability in 2 years of study (Nicolas and Turner, 1993). The advantage of chemical desiccants or senescing agents is that they can be used on irrigated plants or in environments where rainfall is abundant and prevents drought stress. Stress can be imposed at the desired phenological stage and inherent problems arising from differences in phenology and time of stress can be avoided. The objective of this study was to determine the optimum time for spraying with a senescing agent (potassium iodide) to simulate water deficit effects at flowering and grainfilling in pearl millet.

2. Materials and methods

The experiments were conducted during 1989 and 1991 dry seasons (January–May) at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (17°30′N, 78°16′E), India. The soil was an Alfisol (Udic Rhodustalf) of varying depth with ca 75 mm of plant-available moisture in 1989 and ca 45 mm in 1991. Since the dry season is almost rain-free (rainfall 90 mm in 1989 and 72 mm in 1991), with high atmospheric evaporative demand (Fig. 1), the crop was irrigated as necessary except for the drought stress treatment.

In both years, the experiment was conducted as a split-plot design with four replications. The main plots consisted of five treatments: irrigated control; three irrigated treatments with potassium iodide (KI) foliar spray at the boot stage, or at anthesis, or 10 days after anthesis; and a drought stress treatment during flowering and grainfilling. In 1991, KI spray at the boot leaf stage was replaced by KI sprayed at 52 days after emergence (DAE) to make a direct comparison with the drought stress treatment. The subplots consisted of four hybrids, HHB 67, HHB 60, ICH 501 and ICMH 88951, which were machine-sown on ridges 60 cm apart. Each sub plot-unit consisted of five rows (1989) or four rows (1991), of 4 m length. Rows were thinned to 15 cm between plants when the crop was 10 days old. Before planting, 40 kg N ha^{-1} and 18 kg P ha^{-1} as di-ammonium phosphate were banded into the ridges. An additional 40 kg N ha^{-1} as urea was side dressed at 15 DAE. There was no significant damage due to diseases or pests and the plots were kept free from weeds.

In the KI spray treatment, stem and leaves of the crop were sprayed with a 0.3% solution of potassium iodide (99.8% KI active ingredient). This concentration was established after an initial trial and at this concentration the senescence (chlorosis) was gradual. In 1991, chlorophyll content of the combined 2nd and 3rd leaves from the top was estimated at 3-day intervals in the irrigated control, the water deficit and the KI-sprayed treatments commencing from 10 days after anthesis. De-ribbed leaves were shredded into small pieces. Subsamples of 80 mg were placed in 30-ml screw cap bottles, 12 ml of di-methyl sulfoxide added and then incubated at 65°C in a shaking water bath for 3 h. The color intensity in the aliquot was measured at 645 and 663 nm and the total chlorophyll content was estimated as described by Hiscox and Israelstam (1979).

Time to flowering (days) was recorded as when the stigmas had emerged on the main shoot panicles of 50% of the plants in a plot. At maturity, panicles from the central three rows (1989) or two rows (1991) were harvested and dried at 60°C, and grain yield and its components were determined. Data were analyzed by analysis of variance to test the significance of treatment effects and their interactions with hybrids.

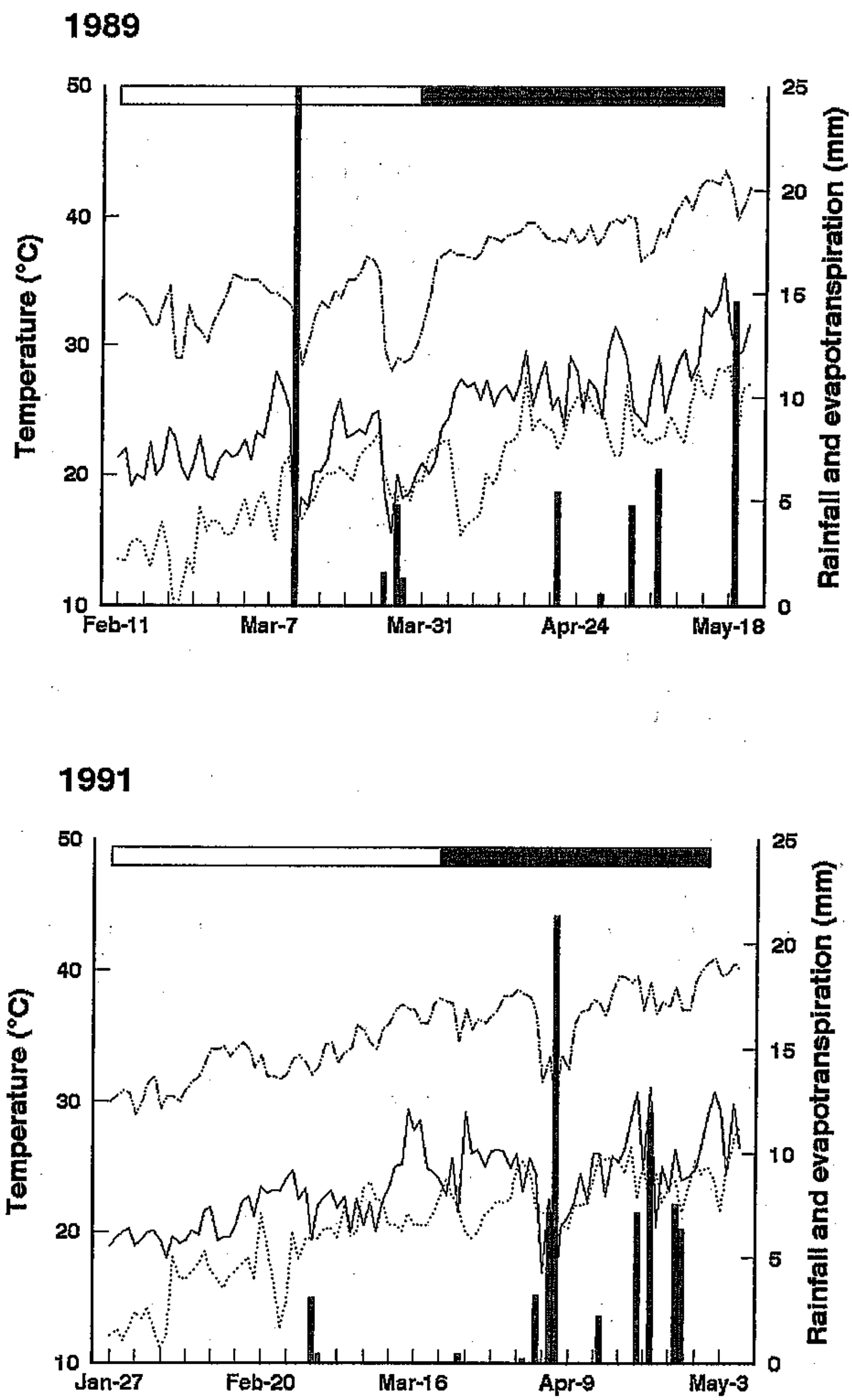

Fig. 1. Daily maximum (—··—··—··) and minimum (———) temperatures, rainfall (vertical bars) and pan-evaporation (····) during the experimental period in 1989 and 1991. The horizontal bar indicates the crop duration and the solid portion indicates the water deficit treatment.

3. Results and discussion

3.1. Effect of season

Mean time to flowering was 45 days in 1989 and 48 days in 1991. In both years, HHB 67 and ICMH 88951 flowered early and the other two hybrids flowered later (Table 1). Mean grain yield in the irrigated control treatment was greater in 1991 than 1989 in all hybrids (Table 1). The hybrid ICMH 88951 had the highest grain yield in both years. The later flowering and higher grain yield in 1991 were due to lower mean temperatures and evaporation rates during grainfilling than in 1989. This was expressed as greater seed size in all hybrids in 1991 (Table 1). The grain numbers were similar in both years and harvest index was higher in 1991.

3.2. Effect of water deficit

Water deficit during flowering and grainfilling reduced grain yield by 38% in 1989 and 67% in 1991 (Table 2, Fig. 2). The greater reduction in grain yields in 1991 than in 1989 was due to reduced available soil water resulting in relatively severe water deficits during the early part of grainfilling. Grain number and grain size were reduced by 22% and 23% respectively in 1989. In 1991, the percentages were 48% for grain number and 35% for grain size. In millet, grain number is reduced more severely by both timing and intensity of stress than is grain size (Mahalakshmi et al., 1987, 1988). Harvest index was reduced by 8% in 1989 compared to 29% in 1991 in the water deficit treatment.

3.3. Effect of senescing agent spray

Spraying of leaves with KI induced a gradual loss of chlorophyll in the leaves though in the water stress plants the loss was even more gradual (Fig. 3). In wheat plants, complete chlorosis of leaves occurred within 2 days of spraying with magnesium chlorate resulting in total loss of photosynthetic capability (Blum et al., 1983b) and senescence (yellowing) occurred within 7–10 days of spraying with potassium iodide (Turner et al., 1989). In wheat, within 3 days of KI treatment, net photosynthesis of the flag leaf was reduced by 85% and transpiration by 50%, and within 7 days these rates were reduced by 99% and 82% respectively (Turner et al., 1989). Chlorophyll content in the KI-sprayed treatment was lower than that of the water deficit treatment (1.45 mg g^{-1} in water deficit vs 0.35 mg g^{-1} in KI spray after 21 days) suggesting that senescence was more severe in KI-sprayed treatments. Water deficit resulted in death of leaves whereas KI treatment led only to chlorosis of leaves. However, grain yield in the water deficit treatment was either similar (water deficit vs KI sprayed at anthesis in 1989) or lower than where KI was sprayed at or after anthesis (1991). This would suggest that despite the faster rate of senescence in KI treatments, the availability and remobilization of assimilates from stems to the grain was higher than in the water deficit treatment. Similar results have been reported in wheat, where reduction in grain size due to KI spray was smaller than that due to drought (Nicolas and Turner, 1993), suggesting that KI affects only current photosynthesis whereas drought affects other processes including initial grain growth

Table 1
Mean time to flowering, grain yield and yield components of the four pearl millet genotypes in the irrigated control treatment in 1989 and in 1991

Genotype	Time to 50% flowering (days)		Grain yield (g m^{-2})		Grain number (# $m^{-2} \times 10^{-3}$)	
	1989	1991	1989	1991	1989	1991
HHB 67	41	44	415	422	52.0	53.2
HHB 60	49	52	356	425	60.3	54.0
ICH 501	42	45	385	430	42.8	40.6
ICMH 88951	48	51	424	467	41.8	44.8
LSD (0.05)	1	2	25	31	3.6	3.3

Table 2
Mean (averaged over genotypes) grain yield, yield components and harvest index of pearl millet crop under irrigation, potassium iodide (KI) spray at different physiological stages and water deficit during grainfilling in 1989 and 1991

Treatment	Grain yield ($g\ m^{-2}$)		Grain number ($\#\ m^{-2} \times 10^{-3}$)		Grain size ($mg\ grain^{-1}$)		HI (%)	
	1989	1991	1989	1991	1989	1991	1989	1991
Irrigated	395	436	49.2	48.1	8.3	9.2	39	45
Water deficit	246	146	38.3	24.9	6.4	6.0	36	32
KI at boot	188	–	28.4	–	6.6	–	28	–
KI at anthesis (A)	238	209	35.6	32.2	6.7	6.5	31	32
KI at A + 10 days	318	302	41.8	41.1	7.7	7.5	36	38
KI at 52 DAE	–	196	–	29.6	–	6.5	–	30
LSD (0.05)	28	23	4.0	3.6	0.4	0.6	2	2

– treatment not included.
DAE, days after emergence.

(cell number). In wheat, grain growth was reduced 23 days after KI treatment (Turner et al., 1989). Spraying with magnesium chlorate resulted in reduced grain growth between 2 to 10 days depending on the cultivar (Blum et al., 1983a), suggesting that KI treatment was less deleterious than desiccants.

In 1989, spraying at the boot-leaf stage resulted in maximum reduction in grain yield (Table 2, Fig. 2). This was due to reduction in both grain number and grain size though the reduction in number was greater than the reduction in grain size. Similar yield component response to severe drought stress before flowering in pearl millet has been reported, although grain size is determined entirely after flowering (Mahalakshmi et al., 1987). Grain size is a relatively more stable yield component than grain number. Accessible assimilates are made available to fill grains to maintain a minimum grain size that ensures future propagation and survival, at the expense of grain number. Mean grain yield was reduced by 60% in 1989 and 48% in 1991 when KI was sprayed at anthesis. This was due to a reduction in grain number of 28% in 1989 and 33% in 1991 and a reduction of grain size of 19% in 1989 and 29% in 1991. In both years, harvest index was also reduced when KI was sprayed at anthesis.

In both years, spraying at 10 days after anthesis resulted in the smallest grain yield reduction. Grain number was reduced by 15% in both years in this treatment but the reduction in grain size was greater in 1991 resulting in greater reduction in grain yield than in 1989. Similar grain yield responses with timing of water deficit after flowering in pearl millet have been reported (Mahalakshmi et al., 1988). Unlike wheat, pearl millet has a relatively short grainfilling period and a large proportion of grain growth is completed by 15 days after anthesis indicating that post anthesis is not the optimum time to spray desiccants or senescing agents to simulate water deficit effects during grainfilling.

3.4. Comparison of KI spray with water deficit treatment

There were significant differences among the various treatments and among the four hybrids in both the years for grain yield and its components (Table 3). Hybrid × treatment interaction was also significant for all of the variables in both years suggesting that the treatments affected the hybrids differently (Table 3). Orthogonal comparisons showed no significant difference between water deficit and KI spray at anthesis in 1989. In 1991, there was no difference between KI sprayed at 52 DAE and KI sprayed at anthesis. However there were significant differences between the water deficit treatment and the various KI spray treatments that were probably due to the very severe nature of the water deficit in 1991. There was a significant negative correlation between time to flowering and grain yield in the water deficit treatment ($r = -0.75$; $P = 0.001$) in 1989, suggesting that the earlier flower-

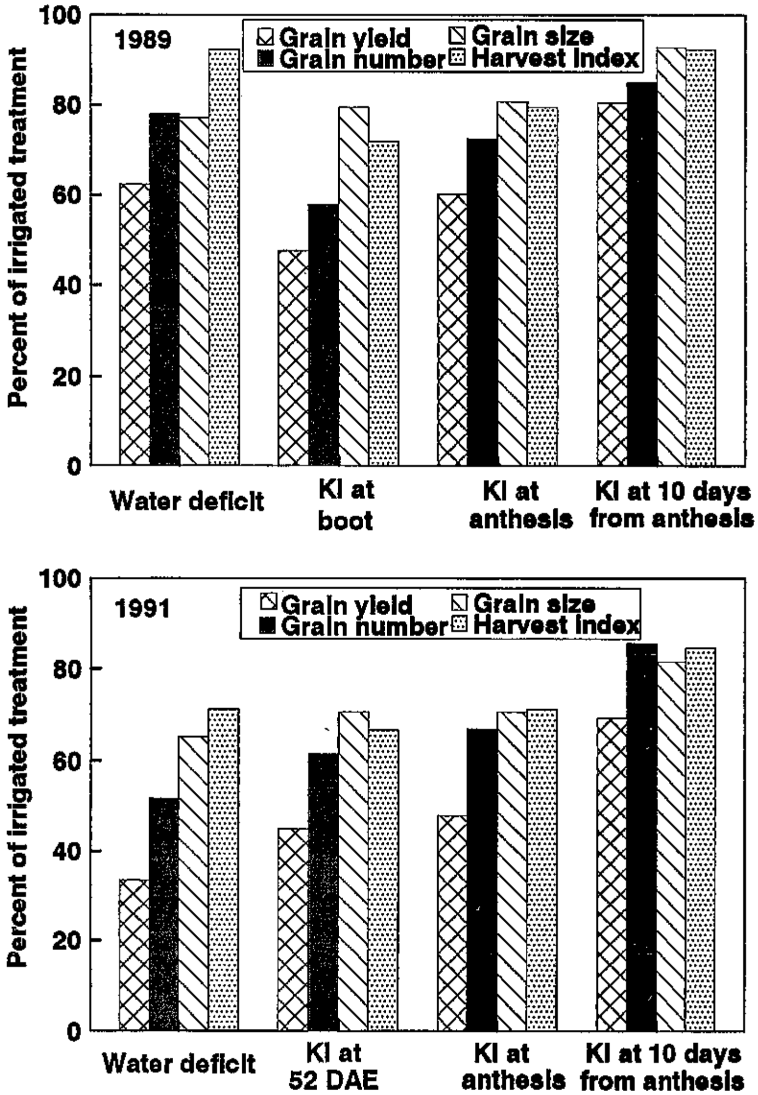

Fig. 2. Mean relative (expressed as percentage of irrigated values) grain yield and yield components, and harvest index in different treatments in 1989 and 1991.

ing hybrids (HHB 67 and ICMH 88951) escaped stress, whereas there was no relationship between flowering and grain yield ($r = -0.46$; $P = 0.08$) in 1991 under relatively severe stress.

There was no relationship between time to flowering and grain yield or its components in any of KI-sprayed treatments suggesting that confounding effects of phenology and timing of stress were avoided in this procedure. In 1989, KI spray at the boot stage affected the hybrids differently with HHB 60 and HHB 67 being affected more severely than the others. These two hybrids have thinner stems than the other two, which probably resulted in smaller stem reserves being available for grain development under assimilate shortage. In wheat, there were differences among cultivars in the amounts of stored assimilates being mobilized under assimilate shortage (Blum et al., 1983b; Turner et al., 1989).

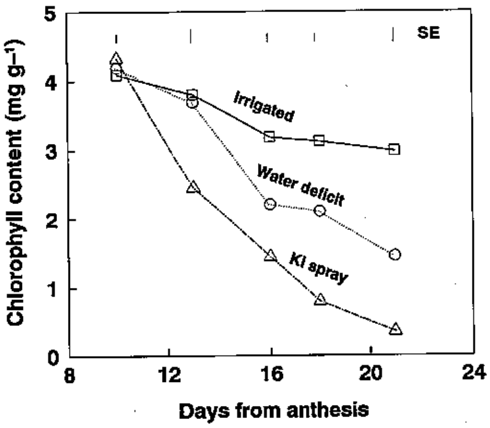

Fig. 3. Mean (HHB 60 and ICMH 88951) chlorophyll content (mg g^{-1} fresh leaf tissue) at different times from anthesis in the combined 2nd and 3rd leaves from top in irrigated control, water deficit and KI sprayed at 10 days after anthesis.

In 1991, KI spray at the boot leaf stage was replaced by KI spray treatment at 52 days after emergence (DAE) to simulate the effect of water deficit beginning at 50 DAE. With KI sprayed at 52 DAE grain yields of ICH 501 and ICMH 88951 were greater than those of HHB 67 and HHB 60. As there was no relationship between grain yield and time to flowering in this treatment and grain yields of the hybrids were similar in the irrigated treatment (Table 1), the higher grain yield of ICH 501 and ICMH 88951 may have been due to a greater contribution by stored assimilates. However, had there been a relationship between time flowering and grain yield, it would not have been possible to separate the effects of phenology in escaping drought, from stem reserve contribution.

In 1991, in another experiment in the same field, 15 cultivars with a wide range of flowering time (42 to 60 days) were grown under irrigated control, KI sprayed at 52 DAE and water deficit beginning 50 DAE. Mean grain yield was 389 g m^{-2} in the irrigated control, 205 g m^{-2} in the water deficit treatment and 173 g m^{-2} in the KI sprayed at 52 DAE treatment. There was no relationship between time to flowering and grain yield in the irrigated control. However, there was a significant negative correlation between time to flowering and grain yield in the water deficit treatment ($r=-0.56$; $P<0.05$) and the KI spray treatment at 52 DAE ($r=-0.67$; $P<0.01$). Clearly, spraying at specific phenological stages would have avoided such confounding effects of phenology with treatments.

Grain yield ranking of the four hybrids was similar in the KI spray at anthesis treatment in both years (Fig. 4). However, grain yield ranking of the hybrids was quite different in the two years in the water deficit treatment. Nicolas and Turner (1993) found that ranking of wheat varieties under a KI treatment was consistent between years. Though they did not compare the ranking of the varieties in the unirrigated treatment in the two years, the grain yield and yield component ranking in the unirrigated treatment was very dissimilar in the two years. This would suggest that the use of senescing agents to simulate photoassimilate shortage was reproducible. The relatively higher grain yields in ICH 501 and ICMH 88951 under reduced current assimilate supply caused by either stress or using a senescing agent suggests that stored assimilates were remobilized to the grains. This aspect needs further investigation.

The grain yield ranking of the hybrids was different in both years when KI was sprayed at 10 days after anthesis. Low temperatures slowed down the grain

Table 3
Analysis of variance (F ratios) for grain yield, yield components and harvest index

Source of variation	d.f.	Grain yield (g m^{-2})		Grain number (# $m^{-2}\times10^{-3}$)		Grain size (mg $grain^{-1}$)		HI (%)	
		1989	1991	1989	1991	1989	1991	1989	1991
Treatment (T)	4	25.0**	96.0**	16.0**	51.0**	14.0**	24.0**	17.0**	21.0**
Hybrid (H)	3	27.0**	15.0**	8.0**	10.0**	137.0**	78.0**	93.0**	70.0**
H×T	12	2.5**	5.3**	2.5**	2.4**	2.5**	2.9**	3.3**	7.7**
CV (%)		14	13	15	15	9	11	9	7

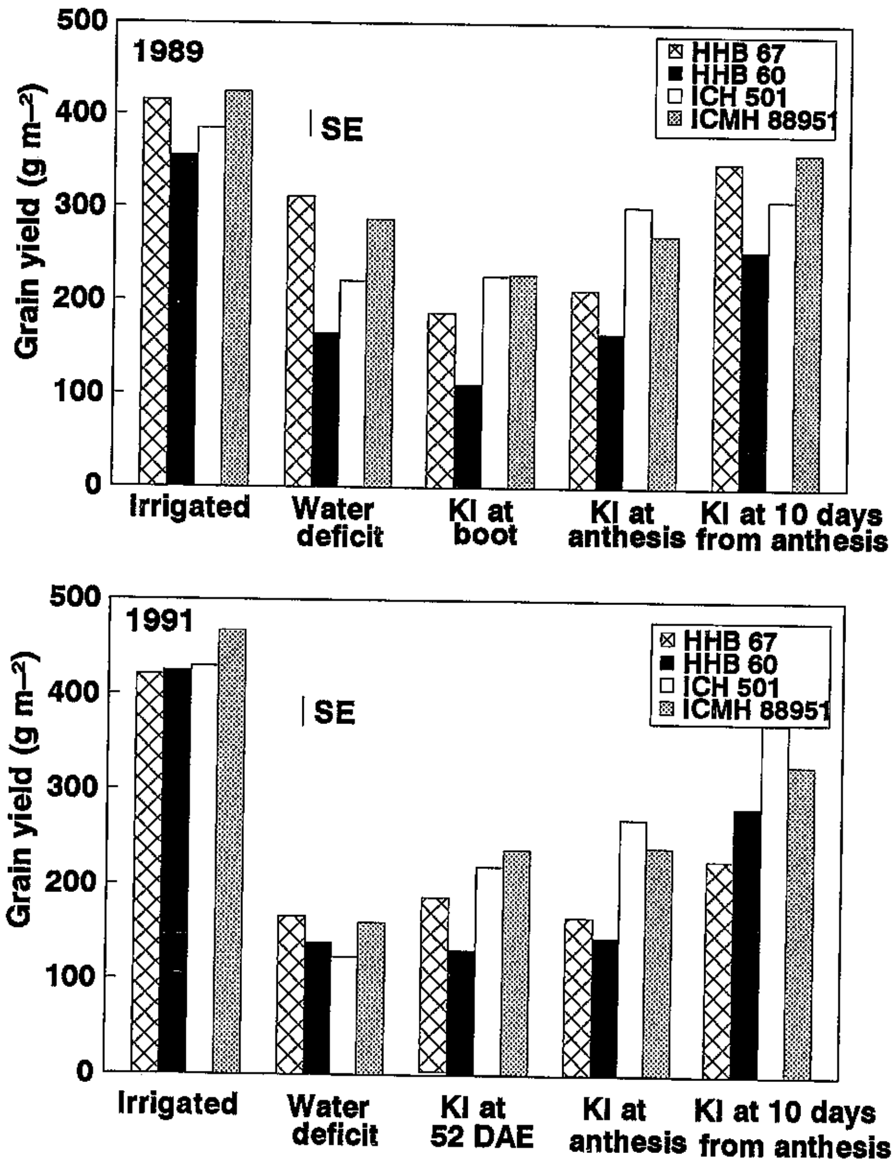

Fig. 4. Mean grain yield (g m^{-2}) of the four hybrids in irrigated control, water deficit and KI sprayed at different times in 1989 and 1991.

growth in early hybrids in 1991 resulting in severe reduction in grain yield when KI was sprayed at 10 days after anthesis but this effect was not observed in 1989.

In conclusion, the reduction of current photosynthesis under water deficit was simulated by spraying pearl millet plants with a senescing agent (KI) in non-stress environments. The optimum time to spray was at anthesis. This technique was more repeatable than dry season water deficit and eliminated the inherent effect of differences in phenology often encountered in empirical field screening methods involving withholding irrigation or in natural stress locations. Sprays with KI would identify lines where stored carbohydrate reserves contribute to grainfilling.

4. References

Bidinger, F.R., Mahalakshmi, V. and Rao, G.D.P., 1987. Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. II. Estimation of genotype response to stress. Aust. J. Agric. Res., 38: 49–59.

Blum, A., Poiarkova, H., Golan, G. and Mayer, J., 1983a. Chemical desiccation of wheat plants as a simulator of post-anthesis stress. I. Effects on translocation and kernel growth. Field Crops Res., 6: 51–58.

Blum, A., Mayer, J. and Golan, G., 1983b. Chemical desiccation of wheat plants as a simulator of post-anthesis stress. II. Relations to drought stress. Field Crops Res., 6: 149–155.

Hiscox, J.D. and Israelstam, G.F., 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot., 57: 1332–1334.

Fischer, R.A. and Maurer, R., 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. Aust. J. Agric. Res., 29: 897–912.

Mahalakshmi, V., Bidinger, F.R. and Raju, D.S., 1987. Effect of timing of water deficit on pearl millet (*Pennisetum americanum*). Field Crops Res., 15: 327–339.

Mahalakshmi, V., Bidinger, F.R. and Rao, G.D.P., 1988. Timing and intensity of water deficits during flowering and grain-filling in pearl millet. Agron. J., 80: 130–135.

Nicolas, N.E. and Turner, N.C., 1993. Use of chemical desiccants and senescing agents to select wheat lines maintaining stable grain size during post anthesis drought. Field Crop Res., 31: 155–171.

Turner, N.C., Nicolas, M.E., Hubick, K.T. and Farquhar, G.D., 1989. Evaluation of traits for the improvement of water use efficiency and harvest index. In: F.W.G. Baker (Editor), Drought Resistance in Cereals. CAB International for ICSU Press, Paris, pp. 117–189.