Morphological Characteristics and Leaf Pigment Contents of Isogenic-diploid and Tetraploid Pearl Millet Inbred Lines

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Pearl millet, *Pennisetum glaucum* (L.) R. Br. is an annual diploid ($2n=14$) species grown primarily for forage in the USA. Autotetraploid pearl millet has been repeatedly induced (Gill *et al.* 1969, Hanna *et al.* 1976, Jauhar 1981) but so far, tetraploids have been of little immediate benefit to pearl millet improvement because of poor seed set. However, potential exists to improve tetraploid pearl millet fertility by increasing heterozygosity (Dujardin and Hanna 1988). In addition, induced autopolyploidy in pearl millet plays an important bridging role in enhancing the crossability with other *Pennisetum* species and in facilitating gene flow (Hanna and Dujardin 1985).

A primary effect of chromosome doubling is an increase in cell size (Stebbins 1950). Induced autotetraploids derived from diploid pearl millets inbred Tift 239DB were characterized by larger stomatal guard cells, larger pollen grains and longer stigmatic hairs on the pistils (Hanna *et al.* 1976).

The expression of induced polyploidy in plant organs has been investigated in a number of cereals, such as tetraploid maize (Randolph *et al.* 1944), rye (Müntzing 1951), sorghum (Schertz 1962), pearl millet (Hanna *et al.* 1976) and *Triticum monococcum* (Kuspira *et al.* 1985).

The effects of ploidy on a number of morphological characteristics in isogenic triploid and hexaploid pearl millet $\times$ napiergrass hybrids showed differential responses among clones (Gonzalez and Hanna 1984). Increased ploidy from $3x$ to $6x$ in these interspecific hybrids had generally a neutral or negative effect on forage yield and quality (Hanna *et al.* 1976).

The existing knowledge of physiological changes associated with genome multiplication is still fragmentary (Lewis 1980). Generally, leaves of tetraploid plants contain less chlorophyll than diploids; however, per unit area, tetraploids are richer in chlorophyll and have higher pigment concentration than their diploid progenitors (Lewis 1980). Warner *et al.* (1987) found that tetraploid pearl millet inbred Tift 239DB had 50% fewer photosynthetic cells per unit leaf area, but twice as many chloroplasts per cell than the diploid isolate, resulting in similar chlorophyll content and photosynthetic enzyme activities on a leaf area basis.

Over the years, there have been numerous studies comparing tetraploids with diploids for various characteristics. However, most previous studies have used a single genotype in a single experiment. The purpose of this research was to develop new tetraploid inbred lines of pearl millet in order to enlarge the tetraploid gene pool and to evaluate the ploidy effects on several morphological and chemical characteristics in three isogenic diploid ($2n=$

14)—tetraploid ($2n=28$) pearl millet inbred lines grown at two planting dates.

Materials and methods

Diploid ($2n=2x=14$) and tetraploid ($2n=4x=28$) pearl millet inbred lines used in this study were 'Tift 23BE' (green plant), 'Tift 239DB' (green plant) and 'Tift 23R' (a red plant due to a dominant gene).

Tetraploid inbreds 23BE and 23R were induced by immersing soaked seed for 6 hours in a 0.1% colchicine solution. Tetraploid Tift 237DB ($2n=28$) has been described previously (Hanna *et al.* 1976). Somatic chromosome counts were made from root-tips pretreated for 2 hours in a saturated solution of monobromonaphthalene, hydrolyzed for 8 min. in 5 N HCl at room temperature and stained in Feulgen's reagent. The three diploid inbred lines and their three tetraploid isolines were planted in the field in 1987.

The experiments were arranged in a randomized complete block design with 5 replications. Plots consisted of 2 rows, each 5 m long with 0.9 m between rows. Plants were thinned to one plant every 0.3 m at three weeks after planting. The test was planted on 26 May, 1987 and 30 June, 1987. The first planting allowed plants to reach anthesis under increasing day lengths while plants in the second planting reached anthesis under decreasing day lengths. Plots received 275 kg/ha 5-10-15 fertilizer at planting.

Data were recorded for 1) number of days to flowering—stigmas exerted on 50% of panicles per plot, 2) plant height—average height from ground to tip of inflorescence on plants in each plot, 3) leaf length and leaf width at the third internode from the top of the plant on 5 plants in each plot, 4) panicle length on 10 plants in each plot, 5) seed weight per panicle from 10 panicles per plot, 6) one hundred seed weight from one plant per plot, and 7) a and b chlorophyll, carotene, and anthocyanin (for 23R) concentrations in the leaf.

Leaf pigment concentrations were determined on the most recently mature leaf of 10 plants per replication of the 30 June planting. The midrib of the leaf was removed and the center third of the leaf was used for all determinations. Leaves were cut into 2 cm square sections, subsampled and leaves mixed, and four 5 g samples withdrawn for determinations. Chlorophyll a and carotene concentrations were determined according to the procedures of Devlin *et al.* (1978). Anthocyanins were extracted and total anthocyanin concentration was determined as developed by Fuleki and Francis (1968a, b).

References to significant is at $P=0.05$.

Results and discussion

The means for number of days to flowering, plant height, panicle length, leaf length, leaf width, seed weight per panicle, and one hundred seed weight for the 26 May and 30 June plantings are given in Table 1. Tetraploid inbreds 23BE and 239DB on the average flowered 4.1 and 3.4 days later, respectively, than their diploid isolines. Although tetraploid 23R tended to flower later than diploid 23R, the small differences were significant at only the 30 June planting date. Each inbred flowered significantly earlier at the 30 June planting compared to the 26 May planting due to the shorter and decreasing day lengths during the flower initiation period of the 30 June planting. No ploidy x planting date interaction was observed, indicating that the same response for days to flowering due to ploidy was observed in both plantings.

Tetraploid inbreds 23BE and 239DB tended to be shorter than their 2x counterparts at both plantings, but the differences were significant only at the 26 May planting. In contrast, 4x inbred 23R averaged 27% taller than its 2x counterpart. Height of 2x and 4x plants of

all inbreds were significantly shorter in the 30 June planting compared to the 26 May planting.

Average panicle lengths of 2x 23BE and 239DB were significantly longer than panicles from 4x plants of the same inbreds. The differences between 2x and 4x plants of these two inbreds were small and probably of little practical significance. However, panicles of 4x 23R averaged 5.2 cm longer than panicles of 2x 23R. Panicles on 23BE and 239DB plants in the 30 June plantings were significantly shorter than panicles from the 26 May planting. The shorter panicles, shorter height and fewer days to flowering of these inbreds in the 30 June planting compared to the 26 May planting are probably due to the onset of shorter days influencing the plant life cycle in the 30 June planting.

Differences in leaf length and width between 2x and 4x plants of inbreds 23BE and 239DB were small and varied between plantings. Tetraploid plants of both inbreds had wider leaves in the 26 May planting than diploid plants. However, a significant differences for leaf length was only observed for 23BE at the 26 May planting when 4x plant leaves were 3.3 cm longer than 2x plant leaves. Large differences were observed for 23R where on the average 4x plants had significantly longer (17.2 cm) and wider (1.05 cm) leaves than 2x plants.

Table 1. Means of the 26 May and 30 June planting dates for days to flowering, height, panicle length, leaf length, leaf width, seed weight per panicle, and one hundred seed weight in isogenic diploid and tetraploid pearl millet

Inbred lines	Ploidy level	Days to flowering	Height	Panicle length	Leaf length	Leaf width	Seed weight/panicle	100 seed weight
				cm			g	
Tift 23BE	2x	50.1b	136a	20.4a	51.1b	2.75a	9.30a	0.64a
	4x	54.2a	122b	18.9b	53.2a	2.82a	0.20b	0.51b
Tift 239DB	2x	67.5b	130a	23.9a	64.2a	3.13b	2.40a	0.38b
	4x	70.9a	119b	23.3b	64.5a	3.41a	0.40b	0.45a
Tift 23Red	2x	73.4a	174b	19.1b	63.1b	2.73b	1.28a	0.39b
	4x	74.4a	221a	24.3a	80.3a	3.78a	1.53a	0.61a

Means followed by the same letter in columns for an inbred line are not significant at $P=0.05$.

Seed weight per panicle was significantly reduced from the diploid to tetraploid level in inbreds 23BE and 239DB due to high ovule abortion. However, tetraploid 23R produced on the average as much seed as its diploid isolate. There was a significant planting date response for seed production per panicle on 23R due probably to more droughty growing conditions during the second planting. A significant planting date x ploidy interaction for seed weight per panicle was observed for 23R. At the 26 May planting, 4x 23R plants set significantly more seed per panicle than 2x 23R plants whereas the reverse was true for the 30 June planting.

Average weight per 100 seeds were significantly higher for 4x 239DB and 23R than for their 2x isolines, whereas the opposite response was observed for inbred 23BE. Increased seed size is usually associated with decreased seed set as observed for 239DB at both planting dates. A significant planting date response for all inbreds is reflected in the reduced seed size in the second planting compared to the first planting. There was also a significant ploidy x planting date response for 23BE and 23R.

Chlorophyll a and b concentrations (Table 2) were similar in both diploid and tetraploid inbreds 239DB and 23R. The tetraploid inbred 23BE, however had significantly higher chlorophyll a concentration than did the diploid.

Carotene concentrations were similar at the diploid and tetraploid level for inbred 239DB. Significantly lower carotene levels were present in tetraploid inbred 23BE than in its diploid

counterpart. Carotene concentration in inbred 23R was approximately three times higher at the tetraploid than at the diploid levels. Anthocyanin weight per gram leaf dry weight also increased by 100% in tetraploid inbred 23R when compared to the diploid. Increase in anthocyanin concentration was reflected in 4x red plants having a darker red color than 2x 23R plants.

Discussion

Tetraploidy in different inbreds of pearl millet produced different results for a number of the characters measured indicating a genotype influence. Similarly, all morphological characters were not affected the same way by an increase in ploidy from the 3x to the 6x level in isogenic triploid-hexaploid pearl millet $\times$ napiergrass hybrids (Gonzalez and Hanna 1984). Although trends for the characters measured were similar for the two planting dates, the differences were more evident in the 26 May planting with favorable growing conditions (good soil moisture, increasing day length, and few leaf diseases) compared to the 30 June planting (drought, decreasing day length, and leaf diseases) indicating a need to have good growing

Table 2. Chlorophyll a and b and carotene optical densities and anthocyanin concentrations in isogenic diploid and tetraploid pearl millet

Inbred line	Ploidy level	Chlorophyll		Carotene	Anthocyanin mg/g leaf dry weight
		a	b		
		OD			
23BE	2x	297b	272a	109a	
	4x	368a	312a	72b	
239DB	2x	361a	299a	108a	
	4x	350a	284a	122a	
23R	2x	310a	273a	35b	50b
	4x	353a	294a	111a	105a

Means followed by the same letter in columns for an inbred line are not significant at $P=0.05$.

Optical density (OD) at 645 nm and 663 nm for chlorophyll a and b, respectively.

Optical density at 440 nm and 666 nm.

conditions to maximize the chances for distinguishing ploidy effects. Significant planting date responses were usually reflected in smaller values for the 30 June planting due to environmental conditions. The few significant ploidy $\times$ planting date interactions observed in this study indicated that ploidy may cause certain characters to respond differently in different environments.

Summary

Inbreds with different ploidy level allow for the study of ploidy-genotype effects on various agronomic, morphological and physiological characteristics. Isogenic diploid ($2n=14$)-tetraploid ($2n=28$) pearl millet (*Pennisetum glaucum* (L.) R. Br.) inbred lines Tift 23BE, Tift 239DB and Tift 23R (red plant) grown at two planting dates were evaluated in replicated tests for polyploidy effects on several morphological characteristics and leaf pigment contents. Tetraploid inbred lines 23BE and 239DB flowered later, were shorter and produced smaller panicles with less seed, but showed small or non-significant differences in length and width, and one hundred seed weight. However, tetraploid inbred 23R averaged significantly higher than the diploid in all characteristics except number of days to flowering and seed weight per

panicle. Chlorophyll a and b were in similar concentrations among diploid and tetraploid inbreds, except for tetraploid inbred 23BE which had higher chlorophyll a concentration. Carotene and anthocyanin concentrations increased by 300% and 100%, respectively, from the diploid to the tetraploid level in inbred 23R. The data indicated that changes in morphological characteristics were due to ploidy, plant genotype, and/or environmental conditions. A 26 May planting date with increasing day lengths was more effective for distinguishing ploidy effects than a 30 June planting date with decreasing day lengths.

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