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RESEARCH PAPER

Physiological characterization of finger millet (*Eleusine coracana* L.) for drought tolerance

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Abstract : A field experiment was conducted to characterize finger millet recombinant inbred lines (RILs) for drought tolerance. A set of 150 RILs with two parents IE 2912 and IE 2885 used were used to characterize for drought tolerance traits such as total leaf area, root length, root volume, moisture retention capacity (MRC) and SPAD chlorophyll reading (SCMR). Parent line IE 2912 was superior to IE 2885 for all the traits and both parents differed significantly for all traits except MRC and SCMR. In mapping population, root traits, leaf traits, showed traits showed normal distribution around the mean and showed continuous variation indicating their quantitative nature. Since identified parental lines and mapping population developed are differing significantly they can be utilized in identifying markers linked to drought traits.

Key Words : Finger miller, RIL, Drought tolerance, SCMR

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INTRODUCTION

Finger millet [*Eleusine coracana* (L.) Gaertn.] subsp. *coracana*, is an important coarse cereal in India and East Africa. The crop is adapted to a wide range of environments, can with stand significant levels of moisture stress. It is grown mainly by subsistence farmers and serves as a food security crop because of its high-nutritional value and excellent storage qualities. Under irrigated conditions in field trials, yields upto 5–6 metric tonnes/ha have been obtained (National Research Council,1996). However, yields in farmers' fields, usually sown with unimproved varieties, are commonly between 1,000 and 2,000 kg/ha.

Finger millet is an important crop grown predominantly under rainfed conditions where drought stress is the major constraint for productivity. Though finger millet is a drought tolerant C_4 species, improving its water acquisition traits and water use efficiency have been shown to be associated with increased productivity under water limited conditions. Identification of genotypes which can with stand the drought condition is very much crucial for enhancing crop productivity for which

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characterizing the genotypes for drought tolerance is important with this background present study was conducted to characterize the finger millet lines for drought tolerance traits.

MATERIAL AND METHODS

During *Kharif* 2005, instead of normal direct sowing, transplanting was taken up for which individual recombinant inbred line along with parents was sown in individual pots. RILs were grown upto 25 days in the pots After 25 days of sowing the seedlings were transplanted in the specialized root structures with a dimension of 10 x 60 sq. ft. (Plate A) in three replications and the individual lines were replicated in a row in each structure. Plant nutrition was taken care by providing N: P: K (50:40:25 kg/ha recommended dose) in two split doses. The transplanted RILs were evaluated for physiological traits such as root traits, shoot traits, leaf traits, SCMR, MRC.

Plate A: RILs grown along with parents in specialized root structures for phenotyping

Two parents IE 2912 and IE 2885 used in this study were selections from *Eleusine coracana* contrasting for drought and neck blast resistance. The parent IE 2912 is resistant for neck blast and drought whereas IE 2885 is susceptible for neck blast and drought. Using these two contrasting parents 150 RILs (recombinant inbred lines) were developed and these RILs were used for both phenotyping and genotyping.

Morpho physiological traits :

Leaf area:

A sample of 5 leaves from one plant was taken and their area was determined by measuring the L x B of each leaf. The ratio of leaf area to the leaf dry weight was computed as specific leaf area. The remaining leaves of a plant were separately oven dried. The dry weight was multiplied with actual SLA to arrive at the total plant leaf area.

Leaf area was measured by length x Breadth method

Leaf area = Length of the leaf in cm x Breadth of the leaf in cm

SLA = Leaf area in cm^2/ leaf dry weight in gram.

Root length and root volume:

The roots were separated from the plants and the root length was recorded using the graduated scale. A known volume of water was taken in a graduated beaker, into this the separated roots were immersed and then the increase in the volume of water was recorded which actually represents the actual root volume.

Tiller number per plant and plant height:

At the time of harvest, numbers of tillers were counted from the plants and the plant height was measured and expressed in centimeter.

SCMR (*SPAD chlorophyll meter reading*):

SCMR (SPAD chlorophyll meter reading) values which are an indication of chlorophyll status in the leaf was measured using SPAD meter. Portable SPAD meter was clamped onto the leaf at different positions as well as on different leaves (3^{rd}, 5^{th}, 7^{th} leaf from top) of the plant and the SPAD reading was measured. The mean of SCMR reading was taken out in the end and presented as average SPAD values.

Moisture retention capacity (*MRC*):

Five leaves from each RIL were detached and immediately fresh weight was recorded, subsequently fresh weight of leaves of all the RILs was recorded at every successive 20 minute interval upto three hours. After three hours, leaves were kept for oven drying for 2-3 days at 60^0C. Using both fresh and dry weights, MRC was calculated using the formula:

$$MRC = \{(FW_1 - DW)/ (FW_i - DW)\} \times 100$$

FWi - Fresh weight immediately after harvest in gram (initial fresh weight)

FW_1 - Weight at a particular hour after harvest in gram

DW - Oven dry weight in gram.

Statistical analysis:

The data obtained from experiments were analyzed using statistical software packages like MSTATC and MS EXCEL, etc. The genotypic variability of physiological traits were assessed using analysis of variance as per Fisher's method. The level of significance was tested at 0.05 and 0.01 probability level in 'F' test. The genotypic means were compared with the critical difference values. This analysis was performed using MSTATC.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Phenotypic evaluation of parental lines :

Based on some previous studies, two *Eleusine coracana* lines IE 2912 and IE2885 were identified as contrasts for drought tolerance and neck blast resistance. The parent IE 2912 was resistant for neck blast and drought whereas IE 2885 was susceptible for both these stresses. Several drought tolerance traits such as root traits including growth parameters were recorded to examine the differences between the parents as well as among the RILs. The parent IE 2912 emerged as the superior parent with higher values recorded for all parameters except SCMR and MRC (Table 1). Significant difference found among parental lines in leaf traits such as total leaf area (3686 cm^2 pl^{-1} in IE 2885 and 5884 cm^2 pl^{-1} in IE 2912 with P=0.005) and leaf weight (15.5 g pl^{-1} in IE 2885 and 23.2 g pl^{-1}in IE 2912 with P=0.002).

Though, tiller number per plant was not significantly different between the parents, stem weight (56.6 g pl^{-1} in IE 2912 and 45.6 g pl^{-1} in IE 2885) and plant height (141.5 cm in IE 2912 and 127.7 cm in IE 2885) varied significantly. The two parents differed significantly in root traits such as root dry weight (P=0.04) and root volume (P=0.006) with parent IE 2912 having the higher root dry weight (16.6 g pl^{-1}), root volume (17.6 cm^3) compared with IE 2885. Root length, root to shoot and root to leaf area ratios were not significant among parents.

Phenotypic evaluation of mapping population :

The mapping population (recombinant inbred lines) was also subjected to physiological characterization in addition to parental lines such as leaf, shoot and root traits along with SPAD chlorophyll meter reading (SCMR), moisture retention capacity (MRC).

Phenotypic variations for leaf traits in the RILs of finger millet:

All the leaf traits like, total leaf area, total leaf weight and SLA showed significant variability among the RILs. The total leaf area varied from 2143 cm^2 $plant^{-1}$ in MLC 51-3 to 8879 cm^2. $plant^{-1}$ in MLC 62-3 with a mean of 4758 cm^2. $plant^{-1}$ (Table 2). The total leaf weight also showed a significant variability ranging from 7.75 g $plant^{-1}$ in MLC 74-3 to 37.06 g.$plant^{-1}$ in MLC 148-1. The ratio of leaf area to leaf weight referred to as specific leaf area (SLA) varied between 135.6 cm^2 g^{-1}

Table 1: Differences in physiological traits among parental lines in finger millet **(n=3)**

Trait	IE 2885	IE 2912	P < 0.05
Total leaf area (cm^2 pl^{-1})	3686.00	5884.00	0.005
Leaf weight (g pl^{-1})	15.50	23.20	0.002
Specific leaf area (cm^2g^{-1})	237.50	254.00	NS
SCMR	47.90	42.90	NS
Stem weight (g pl^{-1})	45.60	56.60	0.002
Plant height (cm)	127.70	141.50	0.005
Tiller number per plant	3.80	4.30	NS
Root length (cm)	30.60	42.10	NS
Root volume (cm^3)	14.40	17.60	0.006
Root dry weight (g pl^{-1})	8.90	16.60	0.044
Root/Shoot	0.145	0.208	NS
Root/LA (g cm^{-2})	0.0024	0.0028	NS
Moisture retention capacity (%)	98.60	97.54	NS

NS=Non-significant

in MLC 85-4and 301.5 cm^2 g^{-1} in MLC 83-1 representing a significant genotypic variability.

All the leaf traits showed normal distribution around the mean and showed continuous variation (Fig.1) indicating that the traits are quantitative in nature. Total leaf area, leaf weight and SLA were positively skewed indicating the predominance of transgressive segregants towards the superior parent for these traits in the population. The sharper kurtosis peaks for LA and SLA (0.55 and 1.37, respectively) indicates that the more segregants had a value much higher than the mean of the population, whereas leaf weight showed negative kurtosis (-0.004).

Phenotypic variations for shoot traits in the RILs of finger millet:

Several parameters associated with shoot growth such as plant height, number of tillers and shoot weight were recorded. The tiller number varied significantly from 1.2 in MLC 71-3 to 6.0 in MLC 68-2 (Table 2). RIL MLC 2-2 showed the lowest stem weight of 31.77 g $plant^{-1}$ and RIL MLC 43-2 showed a highest stem weight of 131.68 g $plant^{-1}$. The mean plant height of RILs was 144 cm ranging from 118.3 cm in MLC 17-3 to 161.6 cm in MLC 41-3 representing a significant variability.

The shoot traits exhibited normal distribution as indicated in Fig. 1. Stem weight (0.41) and tiller number (0.08) were positively skewed whereas plant height was negatively skewed (-0.40). Both stem weight (-0.07) and plant height (-0.23) revealed negative kurtosis whereas tiller number showed positive kurtosis (0.20).

Phenotypic variations for root traits in the RILs of finger millet:

Water acquisition from deeper soil profiles is a function of canopy leaf area and the ability of root traits to harness water from the soil. In the present study, genotypic variability in several parameters associated with the roots was ascertained. The RILs had a mean root length of 44.8 cm and mean root weight was 11.15 g $plant^{-1}$. The lowest root weight of 5.26 g $plant^{-1}$ was noticed in RIL MLC 41-3 and the highest was noticed in MLC 54-4 (24.76 g $plant^{-1}$). The root to shoot ratio also varied significantly from 0.03 in MLC 5-5 to 0.18 in MLC 74-3 (Table 2).

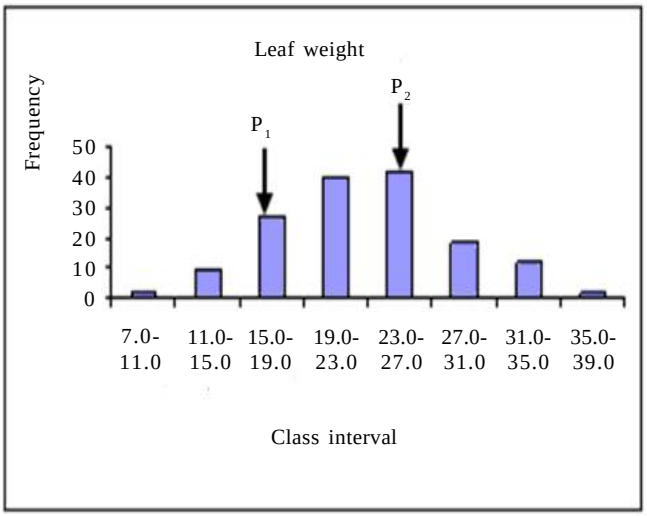
The frequency distribution of root traits showed continuous variability confirming the polygenic inheritance of the trait (Fig.1). Except root length (-0.26) other root traits such as root volume (1.46), root weight (1.17) root to shoot ratio (0.80) and root to leaf area (2.38) were positively skewed with high kurtosis indicating that majority of the recombinant inbred lines performed better than the superior parents for these traits. The kurtosis for root to leaf area (9.54) was maximum among the root traits. Other traits such as SCMR and MRC revealed no significant variability in the mapping population.

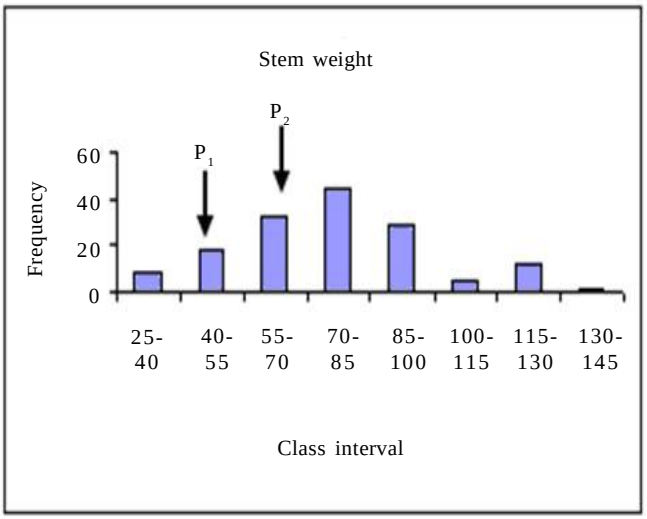
Although significant success was achieved in breeding for yield improvement, most of those efforts were based on selection for yield *per se*. This approach, however, is encountering increasing difficulties in

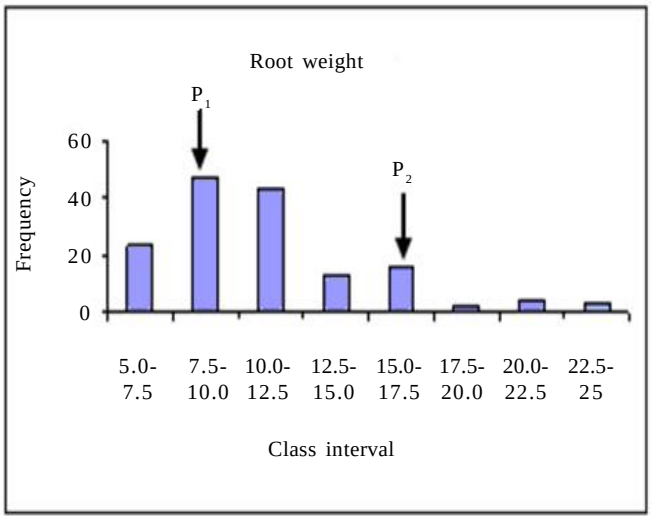
Table 2 : Genetic variability of physiological traits among recombinant inbred lines of the mapping population (IE 2912xIE 2885) in finger millet in first season

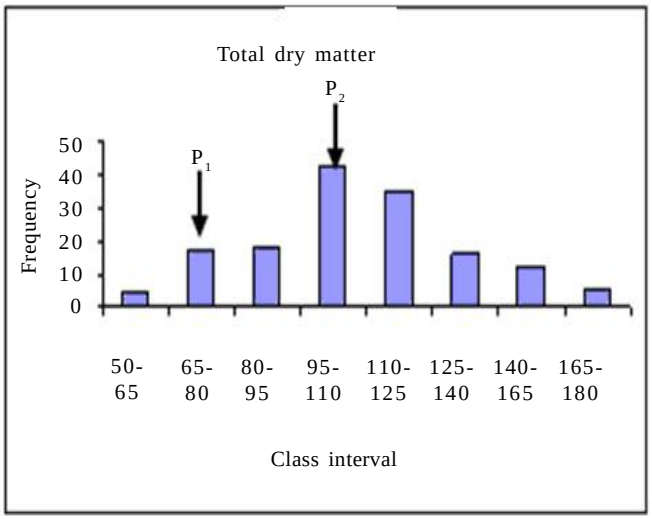
Trait	Minimum	Maximum	Mean	P value	S.E.±	C.D. (P=0.05)	CV %	Kurtosis	Skewness
Total leaf area (cm^2 pl^{-1})	2143.00	8879.00	4758.00	**	10.34	10.80	10.09	0.55	0.60
Leaf weight (g pl^{-1})	7.75	37.06	23.07	**	0.44	0.51	9.77	-0.004	0.12
Specific leaf area (cm^2g^{-1})	135.60	301.50	207.10	**	2.12	3.51	7.47	1.37	0.29
SCMR	40.13	50.48	46.50	NS	0.16	0.68	6.46	0.63	-0.52
Stem weight (g pl^{-1})	31.77	131.68	75.18	**	1.86	1.39	8.17	-0.07	0.41
Plant height (cm)	118.30	161.60	144.00	**	0.73	2.45	7.54	-0.23	-0.40
Tiller number per plant	1.20	6.00	3.85	**	0.07	0.18	21.34	0.20	0.08
Root length (cm)	25.88	63.91	44.80	**	0.67	1.12	11.03	0.20	-0.26
Root volume (cm^3)	6.50	52.50	21.20	**	0.78	0.44	9.18	2.29	1.46
Root dry weight (g pl^{-1})	5.26	24.16	11.15	**	0.30	0.46	18.37	1.42	1.17
Root/shoot	0.03	0.18	0.10	**	0.002	0.004	16.5	0.38	0.64
Root/LA (g cm^{-2})	0.009	0.075	0.024	**	0.007	0.001	21.37	9.54	2.38
MRC (%)	85.30	99.10	96.77	NS	0.12	0.41	1.91	22.52	-3.57

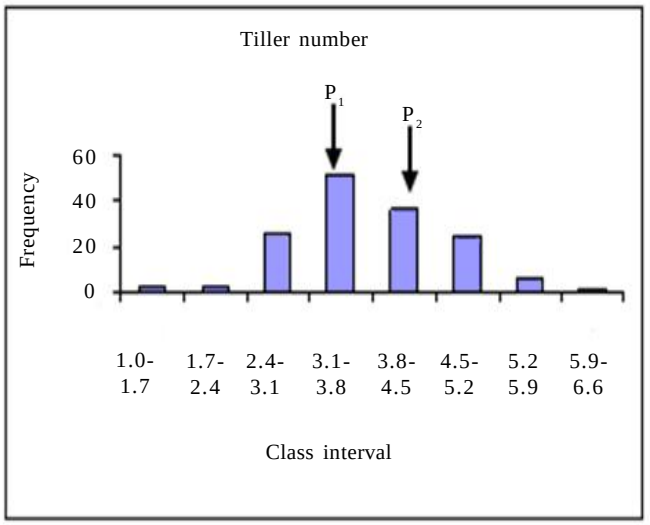
Along with parents mapping population was also phenotyped most of the traits revealed significant genetic variability except SCMR and MRC revealed no significant variability in the mapping population ** indicate significance of value at P=0.05 NS=Non-significant


Leaf weight
Frequency
P1
P2
50
40
30
20
10
0
7.0-11.0
11.0-15.0
15.0-19.0
19.0-23.0
23.0-27.0
27.0-31.0
31.0-35.0
35.0-39.0
Class interval


Stem weight
Frequency
P1
P2
60
40
20
0
25-40
40-55
55-70
70-85
85-100
100-115
115-130
130-145
Class interval


Root weight
Frequency
P1
P2
60
40
20
0
5.0-7.5
7.5-10.0
10.0-12.5
12.5-15.0
15.0-17.5
17.5-20.0
20.0-22.5
22.5-25
Class interval


Total dry matter
Frequency
P1
P2
50
40
30
20
10
0
50-65
65-80
80-95
95-110
110-125
125-140
140-165
165-180
Class interval


Tiller number
Frequency
P1
P2
60
40
20
0
1.0-1.7
1.7-2.4
2.4-3.1
3.1-3.8
3.8-4.5
4.5-5.2
5.2 5.9
5.9-6.6
Class interval

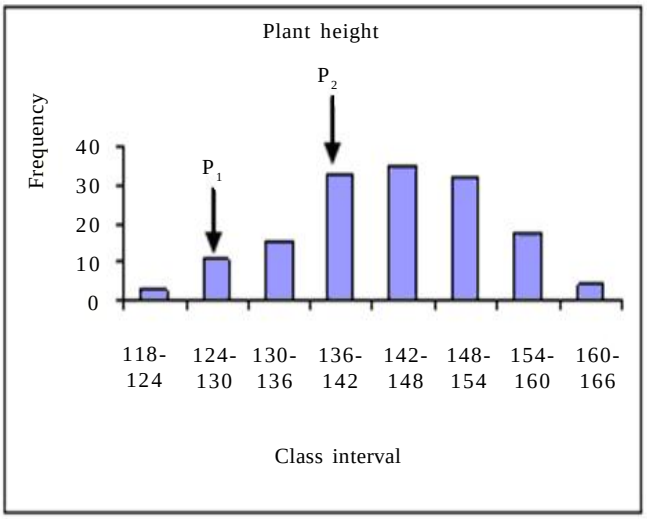
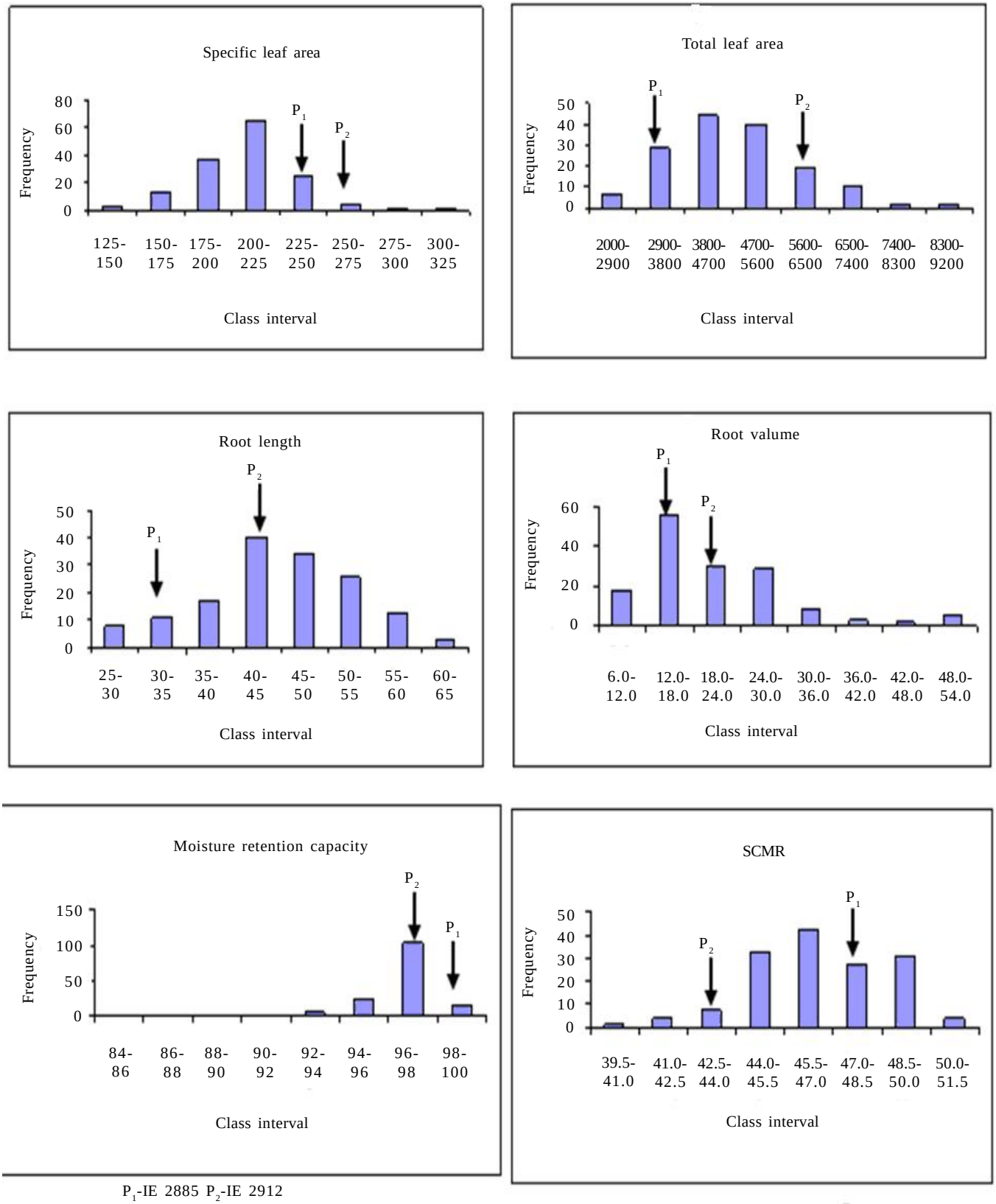

Plant height
Frequency
P1
P2
40
30
20
10
0
118-124
124-130
130-136
136-142
142-148
148-154
154-160
160-166
Class interval

Fig. 1 : Contd.....

Fig. 1 : Contd.....



P_1-IE 2885 P_2-IE 2912

Fig. 1 : Frequency distribution of physiological traits in the first season experiment

achieving further improvement. A narrow variability in yield among the already improved cultivars, a large G x E interaction for yield have often been quoted as the reasons for slow progress in breeding for yield (Araus *et al.,* 2002 and Richards *et al.,* 2002). To achieve further breakthrough in productivity, it is opined that the constituent physiological or morphological traits need to be improved. So the challenge of drought breeding can be addressed through "Trait based breeding approaches".

Most of the physiological traits are quantitatively inherited and bringing together several such traits through conventional breeding would be a formidable task to achieve. Hence, the concept of trait-based breeding would become relevant and achievable, only when robust selection techniques are evolved. One of the most powerful techniques for identifying these desirable individuals from breeding population is DNA based molecular marker. However, before attempting the marker-assisted selection, it is essential to first identify tightly linked DNA markers, with the traits of our interest.

To achieve the envisaged objectives of identifying DNA markers associated with the traits, accurate phenotyping in a suitable mapping population is one of the basic requirements. The construction of a linkage map requires a segregating plant population (*i.e.* a population derived from sexual reproduction). The parents selected for the mapping population will differ for one or more traits of interest. Population sizes used in preliminary genetic mapping studies generally range from 50 to 250 individuals (Mohan *et al.*, 1997), however, larger populations are required for high-resolution mapping. Generally in self-pollinating species, mapping populations originate from parents that are both highly homozygous (inbred). In cross pollinating species, the situation is more complicated since most of these species do not tolerate inbreeding. Many cross pollinating plant species are also polyploid (contain several sets of chromosome pairs).

Mapping populations used for mapping cross pollinating species may be derived from a cross between a heterozygous parent and a haploid or homozygous parent (Wu *et al.*, 1994). Although mapping populations such as F_2 populations and backcross populations are simple and takes less time to construct, but RILs and DH populations are homozygous or 'true-breeding' lines that can be multiplied and reproduced without genetic change occurring. This allows for the conduct of replicated trials across different locations and years indicating their relevance as ideal mapping population.

Thus, a mapping population consisting of RILs derived from cross between IE 2912 and IE 2885 was used in the present study, which was basically developed for blast and drought associated traits. These two parents differed significantly in many traits such as biomass, leaf area, leaf weight, stem weight, plant height, root weight and root volume (Table 1). The same trend was also noticed in mapping population (Table 2). Similar results were reported by O'Leary (1988) in C_4 plant, such as maize. Traits such as leaf area, leaf weight, specific leaf area, stem weight, plant height, tiller number, root weight, root length, root to shoot ratio and root volume varied significantly in mapping population (Table 2) and most of the traits revealed continuous variation suggesting their quantitative mode of inheritance (Fig.1)

In the present study, as the parental lines varied distinctly for drought related traits as well as the developed mapping from the contrasting parental line showed significant differences for drought tolerance traits indicating suitable mapping population with parental lines which can be further used in identifying markers linked drought tolerance traits.

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