Altitudinal Effects on Cytogenetic and Breeding of *Panicum turgidum* Forssk

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Summary Cytogenetic and breeding studies were carried out on *Panicum turgidum* from Saudi Arabia (South west area). The materials were collected from 5 sites and grouped into two main groups according to altitude level. The first group include three sites of collection at sea level, whereas the second one include two sites at altitude of 2600 m above sea level.

Karyotype structure of the first group accessions shows high similarity recording $30m+4sm+2t$. Karyotype measurements of this group recording $1.93\ \mu m$ mean length of chromosome and arm ratio of 1.15 with very low standard error reflect the low variation between chromosomes within the set. In contrast the structure of karyotype in the second group shows $26m+6sm+4t$, with significant variation in chromosome length recording $2.26\ \mu m$ and arm ratio of 1.26. This in addition to the high values of standard error reflect the variation among chromosomes in length and centromere position within the set.

Meiotic behavior of the first group accessions shows a low percentage of disturbed cells compared to that of the second group. In contrast to the second group, normal chromosome association was also observed in the first group accompanied with high level of chiasma frequency. Close relationship was noted between chiasma frequency and regular meiosis in form of chromosome association.

A strong relation was indirectly also noted between chiasma frequency and seed set either in self or open pollination conditions for the two groups. Similarity of karyotype, normal meiosis and high pollen fertility for the first group (sea level altitude) pointed to high stability in genome structure assuming a high potential for self compatibility of the accessions. The second group (2600 m altitude level) shows low fertility associated very strongly with other parameters suggest the partial self-compatible habit at such rich vegetation area which increase the probability of crossing with relatively compatible grasses.

Genus *Panicum* of family Poaceae is of economic importance particularly in subarid zones, providing both low-grade annual cereals and perennial forage species. It is with perhaps over 500 species distributed throughout tropical, subtropical, warm arid regions of the world, and perhaps the largest grass genera.

Although many species of this genus have been subjected to modern criteria of cytogenetic in many sites around the world by many authors (Haroun *et al.* 1992, Haroun 1995, Zuloaga 1991, Rahman 1988), many others are still in need for such modern criteria especially those belonging to middle east area. Previous studies suggest a wide range of chromosome base number of 8, 9, 10 and sometimes 11 (Jauhar and Joshi 1965, De wet and Anderson 1956, Gould 1960, Chen and Hsu 1961). It seems that 9 is the common basic number with diploid, tetraploid and hexaploid levels.

In south west Saudi Arabia (area of study) this genus is represented by 4 species (Migahid 1978). *Panicum turgidum* the most common grass species of this group; grows vigorously at sea level and at Abha site (2600 m above sea level). Following program of study the cytogenetics of grass family Poaceae, *Panicum turgidum* consider a good material not previously studied in this

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area. Modern criteria of cytogenetic and breeding system were investigated in the two levels of altitude.

Materials and methods

Materials of *Panicum turgidum* were collected from their natural habitats and herbarium vouchers were checked for names based on Migahid (1978), and Chaudhary (1989). Collections from 5 sites were grouped into two main groups. The first group represented sea level altitude and included three main sites (along Makkah, Abha Road), whereas the second one represented the altitude of 2600 m above sea level (Abha City, South West Saudi Arabia) and comprised 2 sites of collection.

For karyotype, excised root tips of germinated seeds (1–2 cm long) were pretreated in 0.4% colchicine for 3 h, fixed in 1:3 v/v acetic alcohol for 24 h, hydrolysed in 1 N HCl at 60°C for 8–10 min, and stained in feulgen reagent for 24 h. Fully contracted metaphase cells (up to 5 of 3 to 6 plants for each accession) were used for karyotype study and measurements.

Meiotic behavior and pollen characteristics were studied using young panicles. Buds were fixed in freshly prepared 1:3 solution of acetic alcohol at suitable time. Anthers were collected from prediheciant florets, macerated and squashed in 0.2% aceto-orceine. Meiotic cells were examined for chromosome association and chiasma frequency at diakinesis, metaphase I, and other stages for irregularities.

Pollen fertility was also assessed using the same technique and calculated as a percentage of stained pollen grains.

For seed set study; self and open conditions were compared by enclosing panicles before anthesis using paper bags. To estimate seed set at least 100 florets were examined for each of five individuals. For open pollination panicles dehiscing pollens were placed next to siblings and knocked to release pollen on a number of successive days (Haroun *et al.* 1992).

Results and discussion

Mitotic study

Of the 5 sites studied, accessions of *Panicum turgidum* investigated were tetraploid ($2n=36$), with basic number of 9, which is the most common number among majority of species within this genus (Haroun *et al.* 1992, Jauhar and Joshi 1965). The previous reports on chromosome number of this species are $2n=18$ recorded by Mulay *et al.* (1955), and $2n=6x=54$ by Ferchichi *et al.* (1994), based on $x=9$ as basic number.

Accessions of the first group sites did not differ markedly in mean chromosome length (μm), recording values of 1.96, 1.89 and 1.94 for sites 1, 2 and 3 respectively (Table 1). Mean chromosome lengths of accessions in this group are very close and have very low standard error (± 0.02). This to some extent reflect a degree of homogeneity among chromosome sets of these accessions.

Table 1. Number, total and mean length of chromosomes (chr.l.) and arm ratio (a.r.) for 5 accessions of *P. turgidum* accessions at 2 levels of altitude

Site	2n	Total chr.l. $\pm$ S.E.	$\bar{x}$ chr.l. $\pm$ S.E.	a.r. $\pm$ S.E.
1	36	70.6 $\pm$ 0.9	1.96 $\pm$ 0.08	1.11 $\pm$ 0.09
2	36	68.0 $\pm$ 1.3	1.89 $\pm$ 0.04	10.9 $\pm$ 0.07
3	36	96.8 $\pm$ 1.1	1.94 $\pm$ 0.07	1.12 $\pm$ 0.05
4	36	79.6 $\pm$ 1.7	2.32 $\pm$ 0.11	1.32 $\pm$ 0.11
5	36	83.5 $\pm$ 2.1	2.32 $\pm$ 0.11	1.32 $\pm$ 0.13

Fig. 1. Karyotype structure. a) First group accessions at sea level altitude (30m+4sm+2t). b) Second group accessions at level of 2600 m altitude (26m+6sm+4t). a') Idiogram of first group. b') Idiogram of second group.

The value of arm ratio for these accessions ranged between 1.09–1.12 with mean value of 1.15 ± 0.02 . The low standard error for both chromosome length and arm ratio to some extent prove the previous suggestion for karyotype structure and origin.

Based on length and centromere position karyotype structure of accessions in this group (Fig. 1a, a') shows 15 pairs of metacentric, 2 pairs of submetacentric and one pair seems to be telocentric chromosomes (30m+4sm+2t). In spite of the presence of submetacentric and telocentric types, the majority of chromosomes in the set are similar and homologous in centromeres positions and the average lengths. Such measurements and karyotype structure suggest the autotetraploid origin of the species in the present study (Haroun 1997).

Karyotype structures of the two accessions of sites 4 and 5 (group 2), in contrast to the first group accessions; show more asymmetrical karyotype structure (Fig. 1b, b') with significant variation among chromosomes within the set. Mean chromosome lengths of 2.21 and 2.32 were recorded for the two sites respectively with a mean value of 2.26 for them both which is highly comparable

Table 2. Number of pollen mother cells (PMCs) examined, % of irregular ones, chiasma frequency/bivalent (chia.freq.), % of pollen stainability (poll.stain.), and % of seed set at open and self pollination (O.P., S.P.) for the 5 accession of *P. turgidum* at the 2 levels of altitude.

Site	No. PMCs	% irreg. PMCs	chr. assoc.			Chia. freq./biv.	% poll.stain.	% seed set	
			uni.	bnv.	multi.			S.P.	O.P.
1	15	0.0	0	18	—	1.21	94.6	84.2	90.1
2	17	5.1	0	14	2	1.17	59.1	85.2	93.7
3	15	4.2	1	5	—	1.23	93.4	87.1	90.5
4	14	6.7	2	15	1	1.01	85.1	69.3	77.1
5	16	10.1	3	14	1	1.03	86.7	70.3	75.2

Table 3. Mean values of chromosome length, arm ratio, % of irregularity, chiasma frequency, % of pollen stainability and seed set at self and open pollination condition for the 2 groups of *P. turgidum*

Group	chr.l. $\bar{x} \pm S.E.$	a.r. $\bar{x} \pm S.E.$	% irreg. $\bar{x} \pm S.E.$	chia. freq. $\bar{x} \pm S.E.$	% of stain. $\bar{x} \pm S.E.$	$\bar{x}$ seed set	
						S.P.	O.P.
1	1.93 $\pm$ 0.02	1.15 $\pm$ 0.02	3.1 $\pm$ 1.28	1.20 $\pm$ 0.01	94.4 $\pm$ 0.41	85.5 $\pm$ 0.69	91.4 $\pm$ 0.93
2	2.26 $\pm$ 0.04	1.26 $\pm$ 0.04	8.4 $\pm$ 1.20	1.02 $\pm$ 0.01	85.9 $\pm$ 0.56	69.8 $\pm$ 0.35	76.2 $\pm$ 0.67

with that of first group (1.93). A difference was noted in arm ratio of 1.26 compared with 1.15 for the first group.

Karyotype structure of the two accessions is very close showing 13 pairs of slightly long metacentric, 3 pairs of submetacentric and 2 pairs of telocentric chromosomes (26m+6sm+4t). Compared with the first group, there is few difference in the number of metacentric, submetacentric and telocentric chromosomes and in their lengths. The high values of mean chromosome length, arm ratio and standard error, as well as the variation within chromosomes pointed to the hybrid structure of the karyotype.

To some extend such asymmetrical karyotype and variability within the set in this group is probably due to some chromosomal reorganization within the set, as one could predict that such organization would be accompanied by asymmetrical arm ratio. Based on measurements and variability in karyotype structure it could be assumed that group 2 accessions are under stress of structural hybridity as previously recorded by Haroun (1995) and Jauhar and Joshi (1965) for some species of *Panicum*.

Male meiosis and seed set

Accessions of the first group display a nearly regular meiosis with very low percentage of irregularities ranging between 0.0 to 5.1%, with mean value of 3.1%. Some of them seem to be a result of squash technique as disturbance, breaks and sometimes nondisjunction forms.

Chiasma frequency recorded for these accessions ranged between 1.17 and 1.23 with mean value of 1.20, which is considered significantly high compared with other tetraploid species previously studied within this genus (Chen and Hsu 1961, Jauhar and Joshi 1965). Such high levels of chiasma frequency is probably a consequence of regular meiosis as normal association and the high level of bivalents recorded. The last parameter reflects the high degree of harmony between chromosomes in the set.

No doubt the absence of univalents and low level of multivalents is positively affect chiasma frequency. As it is the site of crossover event where the genetic material exchange takes place, it

could be expected that these accessions have active genes and possess functional gametes leading to increase fertility of pollen (recording 93.4 and 95.1 with mean value of 94.4 as in Tables 2, 3).

Not surprisingly, there is a very close relation between meiotic behavior and pollen stainability. The high level of chiasma frequency can be seen as evolutionary response to the challenge of fertility resulted from regular meiosis. Behavior of chromosomes in meiosis and their characteristics in mitosis pointed to genetic stability and autopolyploid nature of the species in this level of altitude.

Regarding seed set, a strong relationship was also found between pollen fertility and percentage of seed set either at open or self-pollination condition. As the percentage of self-seed set is higher nearly as that of open condition (recording 85.5% against 91.4% for the two conditions respectively), it could be assumed that these accessions are fully self-compatible (Haroun *et al.* 1992). This also suggest that a major component of seed set is pollen fertility.

In contrast, the two accessions of the second group (altitude of 2600 m above sea level), show a significantly high level of irregularities recording 8.4% against 3.1% for the first group (Table 3). However, irregularities observed were previously recorded in many species in this genus and some of related species. There is also a significant reduction in the percentage of pollen stainability recording 85.1% and 86.7% for the two accessions with mean value of 85.9% against mean value of 94.4% for the first group. This insures the strong relationship between meiotic behavior and pollen stainability.

Chromosome association in this group is significantly different from that of the first group, where many univalents (2–3) and low bivalents (13–15) were recorded. Multivalents observed were mostly in form of trivalents and few quadrivalents. No doubt that low chiasma frequency of this group recording 1.02 against 1.20 for the first one which is highly affected by chromosome association (low bivalents and high univalents). In contrast low level of chiasma in this group could be considered as an evolutionary response to the challenge of infertility or partial fertility resulting from multivalents and chromosome association in allopolyploid accession.

Not surprisingly, abnormal meiosis and low chiasma frequency affect synaptic process and drastically reduce the frequency of crossing over. Consequently the gametes formed can not participate in pollination process and so increase the probability of open pollination system. This also insure the characteristics of self incompatibility of the accessions in this group. As there is no great difference in seed set at open and self pollination condition, this could also confirm the probability of cross pollination with some compatible grass or closely related species (Haroun 1995).

Following the results of meiotic behavior no doubt that breeding system in this group is more likely to be partially self compatible, and the heterogeneity of genome structure play an important role regarding this system. It should not be ignored that reduced self pollination in this group also suggest that they may have intermediate partial self incompatibility from another unidentified parent.

As the 5 accessions were divided into two main groups based on altitude level, and found to differ markedly in all parameters investigated, it could be concluded that this factor (altitude level), plays an important role in cytogenetic stability of this species. Finally it should not be ignored that rich vegetation and high diversity of grass species at sites of group 2 accessions, to some extent increase the chance of cross pollination with these accessions. This seems to be the first step of genetic instability and the evolution of the species at this area.

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