ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

EFFECT OF WATER STRESS ON MICROSPOROGENESIS OF A CULTIVATED BARLEY

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Manuscript Info

Manuscript History:

Received: 12 April 2014
Final Accepted: 26 May 2014
Published Online: June 2014

Key words
Barley, chromosomal aberrations, mutagenic potential, water stress

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Abstract

It is well known that most of the genotypic diversity on this earth has resulted from spontaneous variations in the environment and subsequent natural selection. Thus, the mutagenic potential of environmental variations cannot be underestimated. In view of this, a study of mutagenic potentials of environmental variations was conducted, mainly for assessing the effects on the basis of changes in cytological parameters. The environmental variable that was taken into consideration was soil moisture. The soil moisture regime was modified so as to put the plants into stress at three different stages of life history of Barley plant (*Hordeum vulgare* L.) taken as the bioassay. The effect was assessed in terms of chromosomal aberrations in microsporogenesis.

INTRODUCTION

The study of plant environment, which includes, in broad sense, both soil and climatic factors cover a vast field of research. The factors, which might have major influence on growth of plant, are moisture, temperature, photoperiod, soil structure etc. The study of the microclimate and the performance of the plants only provide a picture of the resulting effects brought about by a given changing set of conditions. But the role of individual factors and their delicate interplay in growth regulation cannot be assessed from such studies. However, soil moisture is one of the few factors of plant environment that lend themselves to a considerable degree of control without any elaborate mechanized installation. It is amongst many factors that run counter to the natural establishment, growth and development of the plants. Water is essential in the plant environment for a number of reasons. Water transports minerals through the soil to the roots where they are absorbed by the plant. Water is also the principal medium for the chemical and biochemical processes that support plant metabolism. It also acts as a solvent for dissolved sugars and minerals transported throughout the plant. In addition, evaporation within intercellular spaces provides the cooling mechanism that allows plants to maintain the favorable temperatures necessary for metabolic processes. Water is transported throughout plants almost continuously. There is a constant movement of water from the soil to the roots, from the roots into the various parts of the plant, then into the leaves where it is released into the atmosphere as water vapor through the stomata (small openings in the leaf surfaces).

In relation to plant reactions, it is known that a certain hydration of tissues must be maintained for continuation of active processes at the cell level. Although aging and development of organs may bring about changes in the level of hydration, nevertheless in active cells, the moisture is usually high and therefore, adjustments of moisture balance in soil is of particular interest. Well-watered plants maintain their shape due to the internal pressure in plant cells (turgor pressure). This pressure is also necessary for plant cell expansion and consequently for plant growth. Loss of this pressure occurs due to insufficient water supply.

In subtropical regions of the world where the rainfall is erratic, barley is considered much better than wheat owing to its ability to easily survive water stress (Anjum et al 2003). But even in barley, stress of water also leads to production of an array of stress related chemicals of known and unknown constitutions. These on one hand, help the plant to survive under stress conditions and on the other may have effects on the genes leading to changes in

production of certain hormones or enzymes. Sometimes these changes may inherit themselves or help the progeny to be resistant to such stress conditions.

There has been a large amount of experimental work designed to investigate the response of growth to water regime (Stanhill 1957, Dwyer & Stewart 1987, Reisdorph et al 1999, Anjum et al 2003, Sairam & Tyagi 2004, Tiwari et al 2010, Hameed et al 2011, Fayez et al 2014). However, the results of controlled experiments are difficult to extrapolate in the field performance of any single species (Evans, 1962). Together with this, the cytogenetical correlation of the findings is lacking altogether as far as the available literature is concerned. The present study thus tries to study the cytological effects of water stress conditions taking barley meiosis as a bioassay.

Materials and Methods

For assessment of the effects of soil moisture variations, the barley plants (cv K12) were exposed to moisture stress at 3 stages of growth. These were: -

(1) **Stress 1 (S1)**:8 days of drought at the time of initial seedling establishment ie. Emergence of secondary leaves (20-25 days after sowing).
(2) **Stress 2 (S2)**:8 days of drought at the time of tillering ie. Emergence of tillers (30-35 days after sowing).
(3) **Stress 3 (S3)**:8 days of drought at the time of shooting ie. Multiplication of nodes and increase in internodal length (55-65 days after sowing).

Observations

Table 1 gives a comparative account of cytological behaviour in control and moisture stressed plants of barley. The controls exhibited almost perfect meiosis (0.7% abnormal PMCs) with regular formation of 7II at Metaphase I and normal 7:7 separation at Anaphase I. Although the percentage of anomalies at any stress treatment, was minor in comparison to that of the mutagen treatments but was still significant when compared to the controls. The highest abnormality was induced in case of plants that were stressed at the time of initial seedling establishment (S1), which was 8.54%. This was followed by the abnormities at the tillering (S2) being 5.11%. The lowest percentage of chromosomal anomalies (4.2%) was observed in S3 treatment (stress at the time of shooting). Figures 1-12 present the types of abnormalities encountered during microsporogenesis of stressed plants in comparison to controls.

The types of aberrations were dominated by physiological anomalies like stickiness and clumping at all the phases of division. Stickiness at Metaphase was highest in S1 treatment being 2.03% while clumping was 1.33% in the same treatment. Stickiness at Anaphase was 1.26% in S1 treatment. Stickiness at Metaphase was 1.12% and 0.84% in S2 and S3 treatments respectively.

Secondary association of chromosomes was 0.7%, 0.21% and 0.14% in S1, S2 and S3 respectively. Multivalents were observed in S2 and S3 sets only while univalents were totally absent. Fragmentation was also not observed at all.

Among spindle anomalies, disturbed orientation and late movement of bivalents to the metaphase plate were common. Disturbed orientation was found to be 0.49%, 0.42% and 0.28% at S1, S2and S3 respectively. Late movement of bivalents was 0.63% in PMCs of S1 and S2 while 0.21% in those of S3.

Laggards and bridges were common in Anaphase PMCs. Highest percentage of laggards was encountered at S1 (1.12%) while lowest in S2 set (0.28%). Bridges were also comparatively fewer in S2 and S3 than in S1. Multipolarity was also seen in 0.21% PMCs in S2 and S3 each.

At Telophase, laggards and micronuclei were occasionally present. In case of S2 and S3, shrinkage of PMC size was also observed.

Discussion

Many authors like Schidhalter et al 1998 and Reisdorph et al 1999 have found water stress to be more severe at the seedling stage. Aspinall et al 1964 opines that water stress can reduce tillering capacity and inhibit internodal elongation. Induction of physiological changes in response to conditions of variation in moisture level of soil is quite obvious since the plant tries to survive in adverse conditions by adapting itself. These changes are brought about not only on macro level also on the micro level i.e. cellular level. A reduction in the

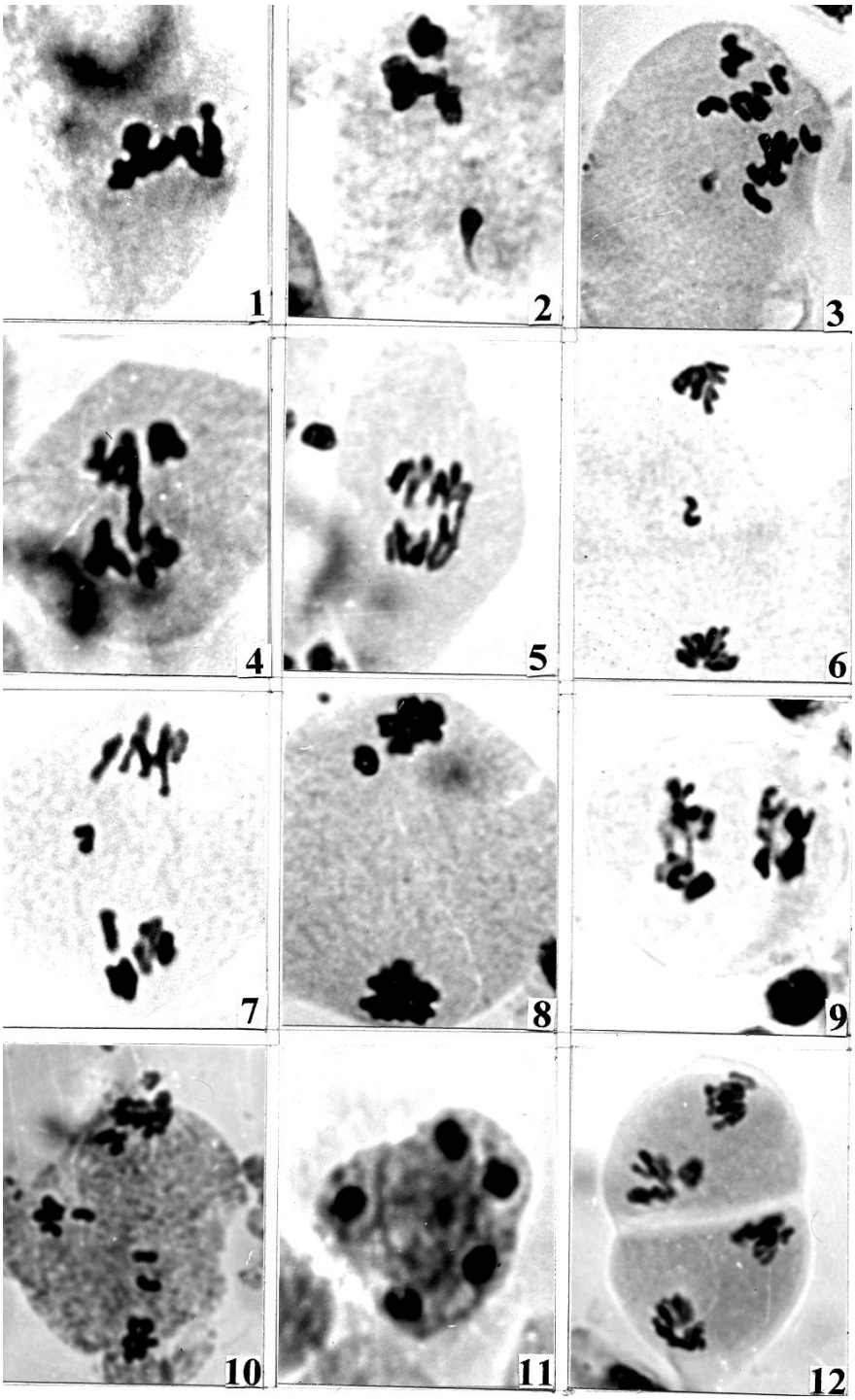
soil moisture content or a variation in the soil composition (through change in nutrient level) causes a condition of stress in all the cell components of which chromosomes and DNA are a part. Under stress, the DNA undergoes slight distortion and thus creates conformational changes in the base structure. The bases under such conditions may behave abnormally. For survival, the plant may even undergo genetic modification thus leading to change in the protein quality and quantity. It may also lead to increase or decrease in various cellular products including sugars and carbohydrates.

Stress, of any kind, has an adverse impact on whole plant morphology, especially when it is given at an early stage. A probable reason seems that since at young stage active division is taking place all over the plant body, any weakness of meristematic cells leads to all weakened daughter cells as in Stress 1 treatment set. In Stress 2 set, plants had grown strong and accumulated sufficient energy for tiller production. This helped them to fight stress and emerge hardened by efficient use of resources. When normal watering was restored, they flourished well and became robust. Perhaps, much genetic variation did not occur as is evident from the low percentage of abnormalities in Stress 2 PMCs. Another reason for the better performance of Stress 2 may be a genetic-environmental interplay, which favoured low moisture levels at tillering. In Stress 3 set, the cytology was badly affected. Since the plants were in their next phase of active division i.e. reproduction, again water was essential for normal formation of reproductive structures and gametes. The results support the findings of Chinoy (1960) on wheat. Studies of Lahiri and Kharbanda (1963, 1966) on drought effects on pearl millet and bulrush millet also provide partial support to the present study.

Most of the cytological abnormalities are indicative of stress effects on chromosomes. Most common abnormality was stickiness, which is physiological anomaly. Jayabalan and Rao (1987) opine that it occurs due to change in cytochemically-balanced reactions. It may also be due to the dissociation of nucleoproteins and alteration in the pattern of organization (Evans 1962), which might have occurred due to reduced cell moisture. Secondary associations observed among bivalents might be interpreted as a result of manifold chromosome rearrangements due to defective duplication, interchanges or stickiness (Stebbins 1950). Multivalents are usually formed due to translocations but they may also result due to stickiness.

Changes in plant environment might have brought about disturbances in cell functioning, leading to distorted formation of spindle fibers which may be the cause of spindle abnormalities like unorientation, precocious movement, early separation and laggards. Unorientation of chromosomes may lead to unequal segregation of chromosomes at Anaphase 1 (Khan 1996). Laggards may also bring about unequal separation. Defective division at first stages of division leads to defects in second phases of division and various abnormalities accumulate to make the gametes sterile. Reduction in yield causes a reduction in seed set and thus, the yield.

The study provides a basis for chromosomal abnormalities caused due to environmental stresses. It can also help in better planning of irrigation. It also brings into forefront the stages of growth, in barley, which are affected the most by water stress or drought.



Figures 1-12: Different types of cytological abnormalities:
1-Stickinss of bivalents; 2-Scattering and clumping of chromosomes; 3-Disturbed polarity at Metaphase I; 4-Anaphase I with sticky bridges; 5-Anaphase I showing late separation; 6-7 Anaphase I with laggard; 8-Abnormal Telophase I; 9-Anaphase II with multiple bridges; 10-Telophase II with many laggards; 11- Multipolarity at Telophase II; 12-Disturbed Telophase II.
(Scale : 1 cm = 4.2 μm)

Table 1: Cytological abnormalities induced in different Stress sets as compared to control set.

Treatment	CF/biv ±SE	Metaphase I/II abnormalities (%)						Anaphase I/II abnormalities (%)					Telophase I/II abn (%)		Other abn (%)	Total abn (%)
		Lm	Do	Mv	St	Cl	Sa	Lg	Br	Ns	St	Mp	Lg	Mn	Sh	
Control	1.80±0.06	-	0.28	-	0.35	-	-	-	-	-	0.07	-	-	-	-	0.70
Stress I	1.69±0.05	0.63	0.49	-	2.03	1.33	0.70	1.12	0.42	-	1.26	-	0.56	-	-	8.54
Stress II	1.77±0.05	0.63	0.42	0.35	1.12	0.70	0.21	0.28	0.14	0.42	0.07	0.21	-	0.14	0.42	5.11
Stress III	1.52±0.09	0.21	0.28	0.35	0.84	0.56	0.14	0.49	0.21	-	0.49	0.21	-	0.14	0.07	4.20

CF/biv=Chiasma frequency per bivalent
Lm=Late movement of bivalents; Do=Disturbed orientation of chromosomes; Mv=Multivalent formation; St=Stickiness of chromosomes; Cl=Clumping of chromosomes; Sa=Secondary association of bivalents; Lg=Lagging chromosomes; Br=Bridge formation between poles; Ns=Non synchronous disjunction; Mp=Multipolarity; Mn=Micronuclei; Sh=Shrinking of PMCs

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