EFFECT OF WATERLOGGING STRESS ON MEIOTIC COURSE, TETRAD FORMATION AND POLLEN FERTILITY OF SESBANIA PEA

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Sesbania cannabina a multipurpose leguminous crop of family Fabaceae, is widely adaptable to adverse climatic conditions such as waterlogging, drought and high salinity. Flooding and water logging are very common phenomena and there may be possibility to become more serious alarms for environment, which is progressively deteriorated by human beings by their anthropogenic activities, polluting the atmosphere. Flooding provides a case of natural selection to the nature which selects the plants which are more adaptable to this condition and renders themselves to survive due to this tolerance or resistance behavior. Present study envisages the effect of waterlogging stress on chromosomal biology of Sesbania pea. To study the effect of waterlogging stress on microsporogensis of Sesbania cannabina, presoaked seeds were sown in experimental pots. Permanent waterlogged condition is created by shifting pots in water filled tanks. Cytological studies showed various types of chromosomal aberrations induced by waterlogging stress and reduction in pollen fertility was also encountered.

Key words: *Sesbania cannabina, waterlogging, cytological studies, chromosomal aberrations, pollen fertility.*

Introduction. Waterlogging refers to a condition when water is present in excess amount than its optimum requirement. Waterlogging occurs when rainfall or irrigation water collects on the soil surface for prolonged periods without infiltrating into the soil. About one third of the world's irrigated areas suffer occasional or more frequent waterlogging [1]. Waterlogging has been shown to limit wheat yields in many regions of the world; an area estimated at 10 million ha is waterlogged each year in developing countries [2]. It creates an anaerobic situation in the rhizosphere due to which the plant experiences the stress (O_2 deficient stress). This O_2 deficiency depresses growth and survival of plants growing in it.

The capacity to survive in flooding condition depends not only on environmental factor, but also on strategy that plants have evolved for adoption to particular flood environments. In flood-tolerant plants, the formation of aerenchyma and adventitious roots in the vicinity of cotyledonary nodes is an indicator of the presence of adaptive mechanisms [3]. The interaction of auxin and ethylene is important for the induction of adventitious root formation [4].

The flooding is supposed to be one of the major biotic influences on distribution of plants worldwide and has led to the emergence of a sizeable minority of modern day taxa with abilities to grow, reproduce and compete strongly in permanently or near permanently flooded environment. Flooding of soil can have a tremendous impact on growth and survival of plants and thereby on agriculture as well as natural ecosystem [5]. In the last decades, considerable progress has been made in our understanding of mechanisms that enable certain plant species and cultivars to withstand periods with excess soil water, or even complete submergence [6]. Various types of histological and physiological mechanisms are known to provide flood tolerance to the plants. Generally there are characteristics of aquatic plants which enable them to grow in aquatic condition. But, leaving this there is some other mechanism which is found to be different from these routine aquatic plants. These mechanisms are furthermore present in few genera and they may be considered as the case of important study for being more tolerant to flood stress as the routine terrestrial plant can tolerate.

There have been many studies of the impact of waterlogging on plant growth, but no work has been done on the cytogenetical behaviour of Sesbania pea due to waterlogging stress. Therefore, the main aim of the present experimentation is to study the effect of waterlogging stress on microsporogenesis and pollen fertility of Sesbania pea.

Material and Methods. To study the effect of waterlogging stress on microsporogenesis of *Sesbania cannabina,* presoaked seeds were sown in experimental pots in a randomized block design at Botany Department, University of Allahabad. The waterlogging stress experiment lasted for one growing season and involved four treatments: a control group of plants was well-watered; second group was

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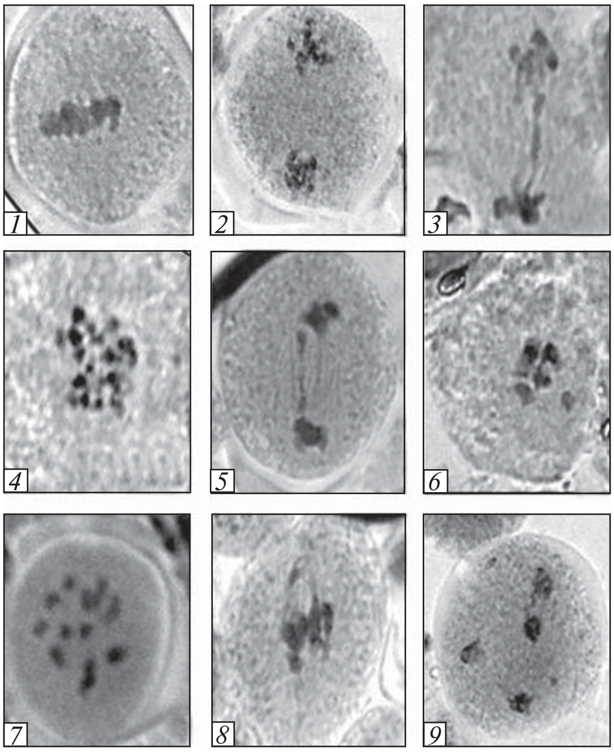
subjected to waterlogging after 15 days of sowing for 10 days, third group was subjected to waterlogging for 20 days and the fourth group was subjected to waterlogging for 30 days. Permanent waterlogged condition is created by shifting pots in water filled tanks. On the onsets of budding young floral buds were fixed in Carnoy's fixative and were transferred in 90 % alcohol after 24 h and used for cytological studies. Slides were prepared using anther squash technique. Anthers were squashed in 2 % acetocarmine. Slides were observed under microscope for different meiotic stages and various chromosomal aberrations, and data were taken for chromosomal aberrations and pollen fertility of each treated set as well as for control.

Results. The result of present investigation clears the chromotoxic behavior of waterlogging stress in Sesbania pea.

Effects of waterlogging stress on microsporogenesis. Wide spectrums of chromosomal aberrations were observed during the metaphasic and anaphasic stages of waterlogging treatment set. Percentages of different types of cytological aberration have been tabulated in Table 1. Control set illustrated perfectly normal behaviour of chromosomes with 12 bivalents at metaphase (Figure, *1*) and 12:12 separations at anaphase (Figure, *2*), whereas in the treated sets substantial increase in abnormality percentage was recorded. A varying degree of chromosomal aberrations (Figure, *3–9*) predominantly occurred due to waterlogging stress treatments.



Pollen mother cells showing various types of cytological aberrations induced by waterlogging stress in *Sesbania pea: 1* – normal metaphase (*n* = 12); *2* – normal anaphase (12:12); *3* – chromatin bridges at anaphase I; *4* – univalent at metaphase I; *5* – laggards at anaphase I; *6* – multivalents at metaphase I; *7* – scattering at metaphase I; *8* – precocious movement at metaphase I; *9* – asynchronization at anaphase II

Various chromosomal abnormalities such as desynapsis at anaphase, univalents at metaphase, scattering of chromosomes at anaphase, asynchronization at anaphase II and laggards at anaphases were present in high frequency, whereas unorientation at metaphase, precocious movements at metaphase, scattering of chromosomes at meta-

Table 1. **Effect of waterlogging stress on meiosis of *Sesbania cannabina***

Water-logging stress	No. of Pmcs observed (mean)	No. of abnormal Pmcs	Metaphasic (I and II) abnormality (%)				Anaphasic (I and II) abnormalities (%)				Others	Total abnorma-lity
			Sc	Pr	Uni	Un	Lg	Sc	Asy	Desyn	%	
Control	200	–	–	–	–	–	–	–	–	–	–	–
10 days	178.66	13.00	0.92	0.55	1.68	0.75	0.92	0.93	0.55	0.93	–	7.27
20 days	219.66	19.00	0.87	1.20	1.51	0.26	1.06	0.28	1.25	2.15	–	8.64
30 days	191.33	23.00	0.32	1.57	1.93	1.22	1.57	1.77	1.77	2.41	0.81	12.07

Note. Sc – Scattering, Pr – Precocious movement, Uni – Univalent, Un – Unorientation, Lg – Laggards, Asy – Asynchronization, Desyn – Desynapsis, Other abnormalities: Multivalents, Tripolarity etc.

phase occurred at lower rate. It was observed that percentage of anaphasic abnormalities was more frequent than the metaphasic abnormalities. A total abnormality percentage was increased with increase in durations of waterlogging stress treatment. Total abnormality percentage registered at 10 days treatment was 7.27 % whereas total abnormality percentage registered at 30 days stress treatment was 12.07 %.

All the chromosomal aberrations were noteworthy at all the durations of treatment. Among all the chromosomal anomalies, desynapsis at anaphase was the most prominent (2.41 %). Followed by it were univalents at metaphase I (1.93 %), scattering of chromosomes at anaphase I (1.77 %), asynchronization at anaphase II (1.77 %), precocious movements at metaphase (1.57 %) and laggards (1.57 %).

Meiotic studies revealed that waterlogging stress was quite efficient and effective in increasing the percentage of abnormal PMCs and desynapsis at anaphase was the most dominant aberration at each treatment set.

Pollen Fertility. Pollen fertility percentages for waterlogging treatment set have been presented in Table 2. In control plants pollen fertility was observed to be 95.50 % and at 30 days treatment of waterlogging stress it was found to 84 %.

Discussion. Many areas all over the world suffer flooding disasters and the restoration of damaged flood plains and stocking of the rivers landscape with flood resistant plants are the topic of active and important research. Therefore, it is important to understand the adaptive responses of plants and effect of these adverse conditions on plant.

Soil O_2 deficiency occurs soon after waterlogging commences, depending on biological activity. With time, waterlogging also changes other soil factors; CO_2, ethylene (C_2H_4), and reduced compounds, such as Mn^{2+}, Fe^{2+}, S^{2-} and carboxylic acids can increase [7]. Internal O_2 transport from air, via the shoots, to roots is essential to survival and functioning of roots [8].

Table 2. **Effect of waterlogging stress on pollen fertility percentages of *Sesbania cannabina***

Waterlogging stress	Pollen fertility percentage, %
Control	95.50
10 days	93.50
20 days	88.66
30 days	84.00

Evaluation of chromosomal variations resulted from the application of waterlogging stress gives an account of mechanism of action of this abiotic stress [9, 10]. These variations lead to various kinds of chromosomal damages, which can be observed as anomalies. Total abnormality percentage was comparatively higher in case of 30 days treatment of waterlogging as compared to 10 and 20 days of stress treatment. Cytological abnormalities such as desynapsis at anaphase, univalents at metaphase, asynchronization at anaphase and scattering at anaphase etc were more frequent in all the treatments.

Precocious movement of chromosomes seems to be a manifestation of improper functioning of the spindle. Unorientation and scattering of chromosomes may either be due to the inhibition of spindle formation or due to the destruction of spindle fibres formed. Desynapsis has been attributed to a variety of causes such as gene action, loss of a chromosome pair, apomixis, structural and numerical changes of chromosomes in addition to environmental causes like, temperature, humidity and soil conditions [11]. During present study, desynapsis appears to have been brought about by waterlogging treatment. Laggards might have appeared due to improper spindle functioning. Higher frequency of chromosomal abnormalities might interfere with the proper tetrad formation which might result into the increase in pollen sterility and subsequent results such as high reduction in yield. Stress treated plants showed high seed sterility which could be attributed to either pollen or ovule sterility or both at the different stages.

Conclusively, waterlogging may have genotoxic effects in a plant which is confirmed by its mutagenic behavior as observed during the present study. The result of present investigation clears the waterlogging tolerance property of *S. cannabina* as it is fully and well adaptable to waterlogging conditions and the physiological processes of plants such as growth, flowering and fruiting and fruit maturity were not retarded by waterlogging and it was found to be a successful survivor in waterlogged condition.

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ВЛИЯНИЕ ЗАТОПЛЕНИЯ НА ТЕЧЕНИЕ МЕЙОЗА, ФОРМИРОВАНИЕ ТЕТРАД И ФЕРТИЛЬНОСТЬ ПЫЛЬЦЫ У *SESBANIA CANNABINA*

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Sesbania cannabina – бобовая культура многоцелевого использования, хорошо адаптирующаяся к различным климатическим условиям, таким как затопление, засуха и высокая засоленность. Затопление – очень распространенный феномен, который может стать более серьезной угрозой окружающей среде, неуклонно ухудшающейся из-за антропогенной активности и загрязнения атмосферы. Затопление является одним из факторов естественного отбора, в результате которого селектируются более приспособленные растения, что позволяет им выживать благодаря своей выносливости или устойчивости. Для изучения влияния затопления на микроспорогенез *Sesbania cannabina* предварительно замоченные семена были высажены в экспериментальные горшки. Условия постоянного затопления создавались при перемещении горшков в заполненные водой емкости. Цитологический анализ выявил различные типы хромосомных аберраций, индуцированных затоплением, и снижение фертильности пыльцы.

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