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Effects of Herbicides on Bajra (*Pennisetum typhoides* Burm.) Stapf.

by

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Introduction: From time to time many workers have reported the mutagenic effect of a wide range of herbicides, fungicides and pesticides when used on crop plants (Liang *et al.*, 1967, Wuu and Grant, 1966, 1967, 1967b). However, effective dosage of the chemical which can control the weeds, pests or diseases may not be sufficient enough to cause chromosomal changes, but in higher concentrations many of these chemicals have been shown to induce chromosomal changes, aberrations and point mutations. Wuu and Grant (1967a, 1967b, 1967c) have investigated on the induction of chromosome aberrations in barley and *Vicia faba*. A better understanding of the cyto-genetic response of plants to herbicides and their relationships to herbicidal performance would lead to the formulation of effective and safe chemical weed control. In the present study, the effect of different herbicides on germination, seedling growth, mitotic index, microsporogenesis and pollen fertility in *bajra* has been reported.

Materials and Methods: Dry seeds of *bajra* var. D_{171} were used in this study. Seeds pre-soaked in distilled water for 12 hrs were treated with 25 ml of acqueous solutions (1000 ppm) of five different herbicides or distilled water for a duration of 12 to 24 hours at room temperature (29°C). Five hundred seeds were used in each treatment. The percentage of active ingredients, concentrations of the chemicals used, their p^H etc., are given below:

Herbicide	% of active ingredient	Concentration used	p^H
Atrazine	50	1000 ppm	9.0
Simazine	50	,,	8.5
2-4-D	80	,,	6.0
Rogue	25	,,	6.0
Tok	46	,,	6.0

The seeds were washed thoroughly in water and sown in seed pans (18"×6") and seedlings were raised. 100 seeds were seperatad from each lot and they were laid out for germination tests in petri-plates under controlled conditions. Fifteen seeds were spread out evenly in each petriplate with a calculated quantity (5 ml) of distilled water. The experiment was replicated four times. About ten seeds were placed for germination in another set of petriplates and the root tips were collected on the third day for mitotic studies.

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Root-tips were fixed in acetic alcohol (1 : 3) and stained with leuco-basic fuchsin and squashed in a drop of aceto-carmine to study the mitotic index. A minimum of 50 cells was scored to work out the mitotic index in each treatment.

Seedlings raised in seed pans were transplanted on the 21st day in the field in 10' long rows, 18" apart with a plant-to-plant distance of 6". Five plants were selected at random in each treatment to study the meiotic behaviour and another set of five plants to study the pollen fertility. Pollen grains were collected at 9.30 a.m. stained with 1 : 1 acetocarmine-glycerine mixture and kept for 3 hours. Five slides were prepared for each plant and from each slide five different fields were scored for fertile and sterile pollen grains.

Results: *Germination:* Germination was recorded from 3rd day and observed through seventh day when there was no increase in the germination in each treatment (Table 1). It was observed that there was reduction in germination percentage with increase in the duration of treatment with 2-4-D, Tok, Atrazine and Simazine. None of the seeds treated with Rogue for 24 hrs germinated. 2-4-D has drastic effect on germination reducing it to 2%. The

TABLE 1. *Germination, seedling growth, pollen sterility and mitotic index in different treatments.*

Treatment	Germination (%)	Root (cm) Mean S.E.	Shoot (cm) Mean S.E.	Pollen sterility (%)	Mitotic index		
					No. of cells studied	Dividing cells	Mitotic index (%)
Control:							
0 hr	53.0	2.68±0.78	2.73±0.55	9.47	581	99	27.04
12 hr	31.0	3.39±1.12	3.15±0.48	6.53	527	79	14.99
24 hr	8.0	3.19±1.07	1.45±0.76	8.31	537	80	14.90
Atrazine							
12 hr	20.0	2.40±1.35	2.70±0.59	41.45	593	66	11.13
24 hr	8.0	1.16±0.62	1.10±0.71	53.06	636	46	7.23
Simazine							
12 hr	20.0	2.98±1.52	2.57±0.99	55.01	529	53	10.02
24 hr	15.0	0.52±0.27	1.59±0.53	62.79	—	—	—
2-4-D.							
12 hr	15.0	0.20±0.22	0.40±0.31	—	523	10	1.91
24 hr	2.0	—	—	—	555	40	7.21
Rogue							
12 hr	13.0	1.36±0.71	2.38±0.67	97.19	505	66	13.07
24 hr	0.0	—	—	71.81	528	81	15.34
Tok							
12 hr	15.0	2.03±2.08	2.08±1.01	72.30	540	52	9.63
24 hr	7.0	1.75±0.26	2.90±0.44	45.93	504	69	13.70

germination percentage in Tok and Atrazine (24 hrs) treated seeds was 7 and 8% respectively, whereas in Simazine treated seeds it was 15%. Germination was reduced by more than 50% with an increase in treatment duration from 12 to 24 hours in Atrazine, 2-4-D, Rogue and Tok. However, fairly good germination was observed in Simazine treated seeds even for a longer duration.

Seedling Growth: The seedlings were measured for their root and shoot growth on the third day from the date of sowing (Table 1). The root growth was better in control than the growth observed in the treated seedlings. Treatments with Atrazine, Simazine and Tok gave similar results showing reduction in root growth by increase in the treatment duration. However, there was complete arrest of seedling growth in 2-4-D and Rogue treatments for 24 hours, though they could allow the roots to grow to a mean length of 0.20 and 1.36 cm respectively in 12 hr duration treatment.

The same trend in shoot growth was observed in Atrazine, Simazine, 2-4-D and Rogue treated seedlings as it was with root growth. In case of seedlings treated with Tok, an increase of 0.82 cm in shoot growth over 12 hrs was noticed in longer duration (24 hrs), as also seen in control (12 hrs).

Mitotic index: Mitotic index was worked out by calculating the percentage of dividing cells to the total number of cells studied (Table 1).

$$\text{Mitotic index} = \frac{\text{number of cells dividing}}{\text{Total number of cells studied}} \times 100$$

Atrazine has reduced mitotic cell divisions with an increase in treatment duration. No root development was noticed in Simazine treatment for 24 hhurs. The results of 2-4-D, Rogue and Tok are quite interesting. These three herbicides have affected the mitotic divisions to a great extent at 12 hr treatment compared to the treatment for longer durations.

Meiotic studies: Five plants selected at random from each treatment were analysed cytologically for chromosomal aberrations in the microsporocytes during meiosis. The flower buds were fixed in acetic alcohol (1:3), the anthers were squashed in a drop of acetocarmine and observations made on temporary slides. A minimum of 50 cells were studied critically in each treatment. The data were recorded (Table 2) regarding the multivalent associations at Metaphasse-I and other abnormalities during disjunction at Anaphase-I. All the cells scored in Rogue and Tok treatments were normal as in control, showing 7 bivalents, during Metaphase-I. But in plants treated with Atrazine and Simazine, multivalent configurations were observed to varying frequencies. The range of quadrivalents was 0-1 in both the treatments. The range of trivalents was observed to be 0-2 in Simazine (24 hr) and that of univalents 0-4 in both the treatments.

TABLE 2. *Frequency and types of chromosome configurations in PMCs in different treatments in bajra*

Treatment	No. of cells studied	Normal cells	Abnormal cells	Quadrivalent		Trivalent		Bivalent		Univalent		Other meiotic abnormalities observed
				Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Control:												
0 hr	50	50	—	—	—	—	—	7	7.0	—	—	Normal
12 hr	50	50	—	—	—	—	—	7	7.0	—	—	Normal
24 hr	50	50	—	—	—	—	—	7	7.0	—	—	Normal
Atrazine												
12 hr	50	50	—	—	—	—	—	7	7.0	—	—	Normal
24 hr	50	46	4	0–1	0.04	—	—	3–7	6.7	0–4	0.08	Two bivalents sticky, Three bivalents rod shaped, One ring of four bivalents in Metaphase-I
Simazine												
12 hr	51	50	1	—	—	—	—	7	7.0	—	—	One bridge in Anaphase-I, Early separation of two bivalents in Metaphase-I, Two laggards in Anaphase-I, Two fragments in Anaphase-I
24 hr	50	48	2	0–1	0.04	0–2	0.06	3–7	6.8	0–4	0.10	
2-4-D												
12 hr	—	—	—	—	—	—	—	—	—	—	—	—
24 hr	—	—	—	—	—	—	—	—	—	—	—	—
Rogue												
12 hr	50	50	—	—	—	—	—	7	7.0	—	—	Normal
24 hr	50	50	—	—	—	—	—	7	7.0	—	—	Normal
Tok												
12 hr	50	50	—	—	—	—	—	7	7.0	—	—	Normal
24 hr	50	50	—	—	—	—	—	7	7.0	—	—	Normal

Pollen Sterility: In Atrazine and Simazine treatments, there was an increase in pollen sterility with the increase in treatment durations (Table 1) However, a reverse trend was observed in treatments with Rogue and Tok. A pollen fertility to the extent of 2 81% and 28.19% was noticed in treatments with Rogue from 12 hours to 24 hours duration respectively. 2-4-D was lethal in its action on the final survival as no plant survived even from 12 hours duration treatment.

Discussion: Radio-mimetic action of chemicals was discovered for the first time by Auerbach and Robson (1946), and Oehlkers (1943), almost simultaneously. Since then considerable interest is evinced to find out the mutagenic action of various chemicals.

With the increased use of herbicides in agriculture, lot of attention is diverted to study the effects of such chemicals on crop plants (Liang *et al*, 1967). Wuu and Grant (1967a and 1967b) have clearly shown that such chemicals can cause chromosomal aberrations both in somatic tissue and during meiosis. In the present study, the bajra seeds treated for 24 hours with all the chemicals had a much lower germination than seeds treated for 12 hours. As regards seedling growth, all the herbicides have reduced root growth by about 50% or more in treatments for 24 hours compared to 12 hours treatment. It is clear from the observations that Atrazine, Simazine and Tok treatments are effective in reducing the rate of growth when treated for long duration while in case of 2-4-D and Rogue treatments for long duration were more drastic in effect.

The results obtained for mitotic index and pollen sterility showed the same trend and they indicate that in case of Atrazine and Simazine, the effect is more with long duration of treatment. But in case of 2-4-D, Rogue and Tok the mitotic index is more in the treatments for longer duration although the pollen sterility has dropped down. Reduction in meristematic cell divisions by the application of herbicides is reported by Gibeault and Skogley (1967). It is known that temporary retardation and complete arrest of growth is due to the injuries to the mitotic apparatus when treated with chemical mutagens. In the present study, about 8% of the meiotic cells in Atrazine (12 hr) and two and four percent in Simazine (12 hr and 24 hr treatments respectively) were aberrant in having chromosomal abnormalities like laggards, fragments, bridges and stickiness in addition to slow rate of growth and reduction in mitotic index.

The experiment has been repeated twice and the results are consistent in showing reduction in germination, seedling growth, mitotic index and pollen fertility.

Summary: Seeds of *bajra*, Var. D_{171} presoaked in distilled water for 12 hours were treated with 1000 ppm acqueous solutions of Atrazine, Simazine, 2-4-D, Rogue and Tok for 12 and 24 hours separately with an additional treatment of distilled water as control.

Germination, seedling growth, mitotic index, and pollen fertility were highly affected in all the treatments (12 and 24 hrs duration). 2-4-D was very lethal as no plant survived even at 12 hrs.

By the results obtained in this experiment, it is very necessary to stress the point that these chemicals bring about reduction in germination, retardation in growth and pollen fertility. Hence preliminary studies regarding the action of such chemicals on crop plants and their residual effect if any, are of paramount importance before advocating the use of such chemicals on the field directly. Also the plant species on which each chemical has to be used with the effective safe dose have to be ascertained.

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