

EFFECT OF 2, 4-D HERBICIDE ON THE STOMATAL CHARACTERISTICS OF WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Herbicides are used for the eradication of weeds. 2,4-D is the post emergence herbicide, commonly used to kill unwanted plants. The present study was carried out to determine the effect of different concentrations of 2,4-D herbicide on stomatal characteristics of flag leaf of wheat (*Triticum aestivum* L.). The field experiment was conducted during the year 2013-14 and 2014-15. The experiment was laid out in a randomized complete block design (RCBD). At tillering stage of wheat, 2,4-D herbicide was sprayed and at milking stage wheat samples were collected to analysed the effect of 2,4-D on stomatal characteristics of flag leaf of wheat. High stomatal length of adaxial epidermis and abaxial epidermis was noted in plots treated with 0 M, 0.01 and 0.03 M concentrations of 2,4-D. Concentration 0.03 M showed highest stomatal width of adaxial epidermis and non-significant effect of different concentrations of 2,4-D herbicide was noted on stomatal width of abaxial epidermis. Maximum stomatal density of adaxial epidermis and abaxial epidermis was observed by application of 0.01 M and 0.03 M concentrations of 2,4-D herbicide. Concentration 0.04 M showed highest stomatal index of adaxial epidermis while 0.01 M and 0.03 M concentrations showed maximum stomatal index of abaxial epidermis.

Key words: 2,4-D, herbicides, wheat, concentrations, anatomy, stomata

INTRODUCTION

An herbicide is a pesticide used to kill unwanted plants. The herbicide causes the anatomical change in plants. Plant biochemical and physiological processes are affected by herbicides (Yang *et al.*, 2006; Warabi *et al.*, 2001; Jung *et al.*, 2004; Ha *et al.*, 2003), causing morphological and anatomical modifications (Kamble, 2007; Guh and Kuk, 1997).

Herbicide application in plants caused considerable changes in the leaf anatomy. Stomatal length, width, number of stomata mm² and stomatal index greatly effected by applications of Isoxaflutole, imazamox, Flurochloridone and Alachlor herbicides (Prakash *et al.*, 1978; Anastasov, 2010a; Anastasov, 2010b; Semerdjieva *et al.*, 2015). Herbicide 2,4-D also caused anatomical variations in leaf, stem, root and petiole of *Cassia tora* (Kamble, 2013). Herbicides like 2,4,5-T, 2,4-D and MCPA in *Stachytarpheta* found to decrease the potassium accumulation in guard cells and also stopped wide opening of stomata (Pemadasa and Jeyaseelan, 1976). Wheat (*Triticum aestivum* L.) is one of the main food crops of Pakistan that leads all agronomic crops with respect to area and production. About one third population depends on wheat for protein and caloric supplies. It contributes to 10.3% in agriculture and 2.2% to GDP (GOP, 2013-14). No information has been available on the effect of 2, 4-D herbicide on stomatal characteristics of crops. This study describe the effect of 2,4-D herbicide on stomatal characteristic in the flag leaves of the wheat.

MATERIALS AND METHODS

The experiment was conducted during year 2013-14 and 2014-15 in a split plot design (RCBD) with three replicate, using five varieties of wheat viz, Punjab-2011, AAS-2011, NARC-2011, Millat-2011 and NARC-2009, treated with four different concentrations (0 M, 0.01 M, 0.03 M and 0.04 M) of 2,4-D herbicide. The herbicide was applied by foliar spray at tillering stage using manual pump. For analysis of stomatal characteristics fully developed flag leaves of wheat were collected at milking (Zadoks scale 70) stage (Zadoks *et al.*, 1974).

Stomatal characteristics of flag leaves of wheat were determined by following the method of Rajendra *et al.* (1977). The epidermis of sample flag leaf was peeled off and transferred into slide along with cover slip. The number of stomata and the number of other epidermal cells were counted at 100X magnification (10 X objective and 10 X ocular) from whole fields of view from each of individual leaf surface using Swift 7000D microscope. The stomatal density was calculated as the number of stomata per square millimeter (Stace, 1965). Stomatal index was

calculated which relates the number of stomata per unit area (S) to the number of epidermal cells per unit area (E) (Salisbury, 1927).

Stomatal index (SI) = $[S / (E + S)] \times 100$.

Stomatal length and stomatal width were measured from five randomly selected stomata in each field of view using an ocular micrometer under microscope (10 X objective and 10 X ocular). The stomatal size was converted to micrometer and then averaged was taken. The data were subjected to analysis of variance using MSTAT-C program and Duncan's multiple Range test was applied to differentiate means (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Wheat varieties showed significant results by the application of 2,4-D in case of stomatal length (μm) on adaxial epidermis of flag leaves during 2013-14 and 2014-15 (Table 1). Maximum stomatal length ($52.79 \mu\text{m}$) was observed in plants treated with 0 M concentration of 2,4-D herbicide while 0.04 M concentration contained lowest stomatal length of about $51.27 \mu\text{m}$. In second growing season (2014-15), different concentrations of 2, 4-D herbicide (0 M, 0.01 M, 0.03 M and 0.04 M) showed non-significant effect on stomatal length (Table 1).

Application of 2,4-D herbicide effected stomatal length of abaxial epidermis. Concentrations of 2,4-D herbicide revealed significant effect during both growing seasons (Table 2). During 2013-14, maximum stomatal length ($58.02 \mu\text{m}$) was noted in plants treated with 0 M ($58.02 \mu\text{m}$) and 0.01 M ($57.51 \mu\text{m}$) concentrations of 2,4-D herbicide. During 2014-15, maximum stomatal length ($58.11 \mu\text{m}$) was noted in plants treated with 0.01 M concentration of 2,4-D herbicide and lowest was found in plants treated with 0.04 M ($55.76 \mu\text{m}$) and 0.03 M ($55.64 \mu\text{m}$) concentrations of 2,4-D herbicide (Table 2). Our results are in accordance with (Anastasov, 2010a; Anastasov, 2010b). They investigated that herbicide application reduced stomatal length. Variability in stomatal length by cultivars was also confirmed from the results of Ferreira *et al.* (2007).

Effect of 2,4-D herbicide was observed on stomatal width of adaxial epidermis of five varieties of wheat during 2013-14 and non-significant ($p \leq 0.05$) results was noted during 2014-15 (Table 3). Plants treated with 0.03 M ($16.16 \mu\text{m}$) concentration of 2, 4-D herbicide showed maximum stomatal width and minimum was noted in plants treated with 0.04 M ($14.84 \mu\text{m}$) concentration of 2, 4-D herbicide (Table 3).

During both growing seasons (2013-14 and 2014-15), application of 2,4-D herbicide showed non-significant effect on wheat varieties in case of stomatal width of abaxial epidermis (Table 4). Our results are in agree with the results of Anastasov (2010 a) who reported that the stomatal width of adaxial epidermis decreased with the high concentration (100 ml/dka) of herbicide oxyfluorfenin in *Nicotiana tabacum* L. The decrease in stomatal width may be due to adversed effect of application of herbicide on physiological events such as respiration and photosynthesis in plant (Anastasov, 2010 a).

Stomatal density was effected by application of 2,4-D herbicide. Genotypes of wheat found to be significantly different from each other in case of stomatal density of adaxial epidermis during both growing seasons (Table 5). The effect of different concentrations of 2, 4-D herbicide was observed during 2013-14 and 2014-15. During 2013-14, plants treated with 0.03 M and 0.01 M 2,4-D showed maximum stomatal density. In next year (2014-15), 0.03 M ($392.00 \text{ number/mm}^2$) concentration had high stomatal (Table 5).

Statistically significant effect was noted in five genotypes of wheat under application of 2,4-D herbicide in term of stomatal density of abaxial epidermis during 2013-14 and 2014-15 (Table 6). Plants treated with 0.01 M 2,4-D herbicide had highest stomatal density while lowest stomatal density was noted with 0.04 M concentration of 2, 4-D herbicide during first growing season. During 2014-15, maximum stomatal density ($424.73 \text{ number / mm}^2$) was noted in plot treated with 0.03 M concentration. Lowest stomatal density was observed with 0.04 M concentration (Table. 6). High concentration of 2,4-D herbicide reduced the stomatal density. Our results are agreed with the results of Anastasov (2010a) and Semerdjieva *et al.* (2015). They stated that stomatal number/ mm^2 was decreased with application of herbicides. Variation in stomatal density among cultivars was also demonstrated from the results of Ferreira *et al.* (2007). It may be due to adversed effect on physiological events such as respiration and photosynthesis (Cali, 2009).

During both the growing seasons (2013-14 and 2014-15), a significant difference was noted in varieties and different concentrations of 2, 4-D herbicide with respect to stomatal index of adaxial epidermis (Table 7). Plants treated with 0.04 M and 0.01 M revealed highest stomatal index significantly followed by 0 M (18.71) and 0.03 M (18.57) concentration of 2, 4-D herbicide respectively during 2013-14. Plants treated with 0.04 M (19.30) 2,4-D had maximum stomatal index and lowest was noted in plants treated with 0.01 M (18.93) 2,4-D during 2014-15 (Table 7).

Table 1. Effect of 2,4-D herbicide on stomatal length of adaxial epidermis (μm) of wheat varieties during years 2013-14 and 2014-15.

2,4-D Concentrations/ Varieties	Year 2013-2014					Year 2014-2015				
	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
Aas-2011	54.67 ^{NS}	55.0	54.33	53.33	54.33 A	51.67 ^{NS}	52.33	52.33	52.33	52.17 B
Punjab-2011	53.56	52.67	53.43	52.78	53.11 B	52.56	50.67	51.11	51.11	51.36 B
NARC-2011	54.22	53.55	52.89	50.89	52.89 B	58.22	57.55	57.89	57.22	57.72 A
NARC-2009	54.11	52.33	52.55	51.56	52.64 B	52.33	52.11	52.55	52.11	52.28 B
Millat-2011	47.33	48.56	48.67	47.78	48.08 C	48.44	49.22	49.67	50.44	49.44 C
Means	52.79 A	52.42 A	52.38 A	51.27 B		52.65 ^{NS}	52.38	52.71	52.64	

Any two means carrying the same letter(s) in a row or column are non-significant at $P=0.05$ by Duncan's Multiple Range Test (DMRT). ^{NS} = non-significant

Table 2. Effect of 2,4-D herbicide on stomatal length of abaxial epidermis (μm) of wheat varieties during years 2013-14 and 2014-15.

2,4-D Concentrations/ varieties	Year 2013-2014					Year 2014-2015				
	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
Aas-2011	59.44 ^{NS}	59.78	57.78	57.68	58.67 A	56.78 b-f	64.44 a	55.78 c-g	55.68 c-g	58.17 A
Punjab-2011	58.67	57.00	56.22	56.44	57.08 A	59.68 b	58.33 bc	57.22 b-e	57.44 b-e	58.17 A
NARC-2011	58.34	58.67	57.67	56.67	57.83 A	58.00 bcd	58.44 bc	57.33 b-e	56.33 b-g	57.53 A
NARC-2009	57.89	56.44	56.33	56.89	56.89 AB	54.44 d-g	53.33 fg	53.22 g	53.89 efg	53.72 B
Millat-2011	55.78	55.67	54.33	55.22	55.25 B	56.00 c-g	56.00 c-g	54.66 d-g	55.44 c-g	55.53 AB
Means	58.02 A	57.51 A	56.47 B	56.58 B		56.98 AB	58.11 A	55.64 B	55.76 B	

Any two means carrying the same letter(s) in a row or column are non-significant at $P=0.05$ by Duncan's Multiple Range Test (DMRT). ^{NS} = non-significant

Table 3 Effect of 2,4-D herbicide on stomatal width of adaxial epidermis (μm) of wheat varieties during years 2013-14 and 2014-15.

2,4-D Concentrations/ varieties	Year 2013-2014						Year 2014-2015			
	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
Aas-2011	15.89 ^{NS}	16.22	16.33	15.89	16.08 A	15.33 ^{NS}	17.00	17.11	17.67	16.78 ^{NS}
Punjab-2011	15.33	15.33	15.45	14.55	15.17 C	17.56	16.44	16.45	17.55	17.00
NARC-2011	15.00	16.22	16.67	14.33	15.56 BC	17.00	16.22	16.33	15.67	16.31
NARC-2009	16.33	16.00	16.00	14.55	15.72 AB	14.67	16.00	18.33	16.22	16.31
Millat-2011	15.78	16.11	16.33	14.89	15.78 AB	16.78	15.78	17.33	17.56	16.86
Means	15.67 B	15.98	16.16 A	14.84 C		16.27 ^{NS}	16.28	17.11	16.93	

AB

Any two means carrying the same letter(s) in a row or column are non-significant at $P=0.05$ by Duncan's Multiple Range Test (DMRT). ^{NS}= non-significantTable 4 Effect of 2,4-D herbicide on stomatal width of abaxial epidermis (μm) of wheat varieties during years 2013-14 and 2014-15.

2,4-D Concentrations/ varieties	Year 2013-2014						Year 2014-2015					
	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	Means
Aas-2011	25.11 ab	24.78 a-c	25.67 a	24.89 a-c	25.11 A	21.44 ^{NS}	22.78	23.67	21.22	22.28 A		
Punjab-2011	23.11 ef	24.58 a-d	23.33 def	24.11 b-f	23.78 B	20.44	21.22	20.66	21.44	20.94 B		
NARC-2011	24.95 abc	24.56 a-d	24.33 a-f	23.63 c-f	24.37 AB	20.33	21.56	21.66	21.89	21.36		
NARC-2009	24.22 b-f	23.03 f	23.29 def	23.89 b-f	23.61 B	21.55	20.89	21.00	21.44	21.22 B		
Millat-2011	24.33 a-f	25.11 ab	25.11 ab	24.44 a-c	24.75 A	18.33	18.78	19.11 [*]	18.11	18.58 C		
Means	24.35 ^{NS}	24.41	24.35	24.19		20.42 ^{NS}	21.05	21.22	20.82			

Any two means carrying the same letter(s) in a row or column are non-significant at $P=0.05$ by Duncan's Multiple Range Test (DMRT). ^{NS}= non-significant

Table 5. Effect of 2, 4-D herbicide on stomatal density of adaxial epidermis (number /mm²) of wheat varieties during years 2013-14 and 2014-15.

2,4-D Concentrations/ varieties	Year 2013-2014					Year 2014-2015				
	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
Aas-2011	358.33 ^{NS}	368.33	373.33	368.33	365.83 C	386.67 c-e	386.67 c-e	393.33 b	390.00 b-d	389.17 B
Punjab-2011	366.67	375.00	373.33	371.67	371.67 B	383.33 e-g	384.67 d-f	386.67 c-e	390.00 b-d	386.17 BC
NARC-2011	363.33	376.67	373.33	370.00	370.83 B	383.33 e-g	378.33 g	388.33 b-e	391.67 bc	385.42 BC
NARC-2009	373.33	380.00	381.67	376.67	377.92 A	380.33 fg	373.33 h	391.67 bc	388.33 b-e	383.33 C
Millat-2011	376.67	383.33	386.67	381.67	382.08 A	393.33 b	391.67 bc	400.00 a	398.33 a	395.83 A
Means	367.67 C	376.67 A	377.67 A	372.67 B		385.33 B	382.93 C	392.00 A	391.67 A	

Any two means carrying the same letter(s) in a row or column are non-significant at P= 0.05 by Duncan's Multiple Range Test (DMRT). ^{NS}= non-significant

Table 6. Effect of 2, 4-D herbicide on stomatal density of abaxial epidermis (number/mm²) of wheat varieties during years 2013-14 and 2014-15.

2,4-D Concentrations/ varieties	Year 2013-2014					Year 2014-2015				
	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
Aas-2011	408.33 ^{NS}	420.67	423.33	415.00	416.83 A	428.33 bc	433.33 ab	431.67 b	425.00 cd	429.58 B
Punjab-2011	411.67	417.67	408.67	405.00	410.75 AB	420.00 d-f	415.00 e-g	420.00 d-f	413.00 fg	417.00 C
NARC-2011	406.67	405.00	410.00	399.00	405.17 B	420.33 de	416.67 e-g	416.33 e-g	415.00 e-g	417.08 C
NARC-2009	410.67	410.33	407.33	402.67	407.75 B	419.67 d-g	412.67 g	416.33 e-g	416.67 e-g	416.33 C
Millat-2011	412.33	419.00	418.00	416.67	416.50 A	431.67 b	435.00 ab	439.33 a	425.00 cd	432.75 A
Means	409.93 BC	414.53 A	413.47 AB	407.67 C		424.00 A	422.53 A	424.73 A	418.93 B	

Any two means carrying the same letter(s) in a row or column are non-significant at P= 0.05 by Duncan's Multiple Range Test (DMRT). ^{NS}= non-significant

Table 7. Effect of 2,4-D herbicide on stomatal index of adaxial epidermis of wheat varieties during years 2013-14 and 2014-15.

2,4-D Concentrations/ varieties	Year 2013-2014						Year 2014-2015					
	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	Means
Aas-2011	18.85 ^{NS}	18.95	18.75	19.00	18.89 A	19.41 ^{NS}	19.33	19.34	19.41	19.37 A		
Punjab-2011	18.69	18.80	18.53	18.75	18.69 B	19.16	18.82	19.16	19.31	19.11 B		
NARC-2011	18.59	18.85	18.43	18.75	18.65 BC	18.86	19.58	19.18	19.24	18.97 B		
NARC-2009	18.43	18.59	18.27	18.75	18.51 C	19.03	18.61	18.87	19.11	18.91 B		
Millat-2011	19.00	19.06	18.85	19.16	19.02 A	19.35	19.28	19.46	19.44	19.39 A		
Means	18.71 B	18.85 A	18.57 C	18.88 A		19.16 A	18.93 B	19.20 A	19.30 A			

Any two means carrying the same letter(s) in a row or column are non-significant at P= 0.05 by Duncan's Multiple Range Test (DMRT).^{NS}= non-significant

Table 8. Effect of 2,4-D herbicide on stomatal index of abaxial epidermis of wheat varieties during years 2013-14 and 2014-15.

2,4-D Concentrations/ varieties	Year 2013-2014						Year 2014-2015					
	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	Means
Aas-2011	20.22 ^{NS}	21.15	21.08	20.60	20.76 ^{NS}	19.79 ^{NS}	20.25	20.17	19.83	20.01 ^{NS}		
Punjab-2011	19.58	21.30	19.27	19.08	19.81	19.70	19.92	19.78	19.74	19.79		
NARC-2011	20.31	19.92	20.50	19.83	20.14	19.80	19.70	19.86	19.49	19.71		
NARC-2009	19.45	23.13	20.55	19.96	20.77	19.66	19.56	19.55	19.63	19.60		
Millat-2011	20.10	20.80	22.03	21.51	21.11	19.81	20.06	20.09	19.82	19.95		
Means	19.93 ^{NS}	21.26	20.68	20.20		19.75 B	19.90 A	19.89 A	19.70 B			

Any two means carrying the same letter(s) in a row or column are non-significant at P= 0.05 by Duncan's Multiple Range Test (DMRT).^{NS}= non-significant

All factors (except concentrations of 2,4-D herbicide during 2014-15), showed non-significant ($p \leq 0.05$) results during both growing seasons in terms of stomatal index of abaxial epidermis (Table 8). During 2014-15, significant effect was noted with the application of 2,4-D herbicide. The plants treated with 0.01 M (19.90) concentration showed maximum stomatal index and minimum was noted in plants treated with 0.04 M (19.70) concentration of 2,4-D herbicide (Table 8). Prakash *et al.*, (1978) investigated that the applications of flurochloridone and alachlor herbicides reduced stomatal index.

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