World Journal of Agricultural Sciences 2 (3): 333-339, 2006
ISSN 1817-3047

Stability Analysis of Roselle Cultivars (*Hibiscus sabdariffa* L.) Under Different Nitrogen Fertilizer Environments

[1]M.E.S. Ottai, [1]K.A. Aboud, [1]I.M. Mahmoud and [2]D.M. El-Hariri

[1]Genetics and Cytology Department,
[2]Representative of Eur. Coop. FAO Flax and Other Bast Fiberous Plants
Research Network for Near/Middle East, Field Crops Reseach Department,
National Research Centre, El-Tahrir St., Dokki, Cairo, Egypt

Abstract: Stability parameters were analyzed for five quantitative characters of three reselle *(Hibiscus sabdariffa L.)* cultivars: Sudani, Masri and White under seven nitrogen fertilizer treatments (environments) during two successive seasons i.e. 2003 and 2004. All analysis of variance models showed that cultivars had significant responses toward different environmental changes. Significant cultivar-environmental interactions were detected for most of roselle traits during both seasons. Dried and fresh sepals yield per plant (DSY and FSY) were more sensitive in responsible to environmental changes. According to stability parameters, Sudani cultivar was more stable for DSY, FSY and number of bolls per plant (NB); Masri cultivar was stable for plant height (PH) while White cultivar was stable for number of primary branches per plant (NPB) in the first season. However, White cultivar was stable for all traits except NPB which was stable for Sudani cultivar in the second season. Selection index showed that 20 m^3/feddan of chicken manure followed by 200 kg ammonium sulfate gave the highest nitrogen fertilizer environments and thus they have must recommended for higher yield of roselle plants.

Key words: Stability · roselle (*Hibiscus sabdariffa* L.) · cultivars · nitrogen fertilizer · environments

INTRODUCTION

Stability study for plant genotypes under different environments is an important subject in breeding programs of medicinal plants. Phenotypic performance of a genotype could be altered under diverse agro-environmental conditions. Also, genotypic-environmental interactions have been assumed of greater importance in plant breeding, as they affect the stability of genotypic values under diverse environments [1].

Roselle plant (*Hibiscus sabdariffa* L.) is one of the famous medicinal plants with various medical uses, in addition to its diverse benefits. Sudani, Masri and White are the most three dominant roselle cultivars grown in Egypt [2]. Nitrogen fertilization is an important source for roselle cultivars to increase and improve sepals yield. Cultivars may have different response to nitrogen fertilization rates and types (environments). Therefore, extensive work is required to identify the more stable cultivar with maximum dried sepals yield and quality in relation to more response to suitable nitrogen fertilizers of roselle cultivars. Many authors studied the response of environmental changes on Malvaceae plant genotypes [3-6].

Statistical analysis methods have been proposed to evaluate stability according to genotype-environmental interactions. Eberhart & Russell [7] and Freeman & Perkins [8] presented two models of stability analysis using regression coefficient (b_i) and deviation from regression (S^2_{di}). While Perkins and Jinks [9] used regression coefficient (B_i) only in their model of stability. In addition, selection index analysis is considered to determine the most suitable environment for particular cultivar [10].

The present investigation aims to study (a) genotype, environment and genotype- environmental interaction; (b) stability parameters; (c) selection index of sepals yield and its components in the roselle cultivars.

MATERIALS AND METHODS

Materials: Seeds of three roselle cultivars; Sudani, Masri and White were obtained from the Breeding Program of Genetics & Breeding of Medicinal and Aromatic Plants

Corresponding Author: Dr. K.A. Aboud, Genetics and Cytology Department, National Research Centre, El-Tahrir St., Dokki, Cairo, Egypt

Group, Genetics & Cytology Dept., National Research Centre (NRC), Egypt.

Experimental application: The seeds were sown at Salah El-Din, Village, South of Tahrir, Behira Governorate on April for two successive seasons 2003 and 2004. Seven nitrogen fertilization treatments were applied to the experimental land as the following:

t_1- Without any nitrogen fertilizers as a control treatment
t_2- 100 kg ammonium sulfate/feddan
t_3- 200 kg ammonium sulfate/feddan
t_4- 20 m^3 chicken manure/feddan
t_5- 30 m^3 organic manure /feddan
t_6- t_2 plus t_5
t_7- t_3 plus t_4

A Randomized Complete Block Design with three and five replication was used for the first and second seasons, respectively. Each replicate had five and three lines in the same respecting. The line was 4 m in length and 60 cm in between. The distance between hills was 40 cm and each hill was thinned at one plant. Recommended cultural practices were applied throughout the growing season. Field observations were recorded on three randomly chosen plants for each line. At maturity, the chosen plants were harvested separately and average values of five quantitative characters for each replicate (3 × 5 or 5 × 3 plants) were estimated and considered to statistical analysis. The five studied traits were:

1. Plant height (PH), (cm)
2. Number of primary branches/plant (NPB)
3. Number of bolls/plant (NB)
4. Fresh sepals yield/plant (FSY), (g)
5. Dried sepals yield/plant (DSY), (g)

Statistical procedure: A combined analysis of variance over all treatments (environments) was done for all characters according to Mcintosh [11]. Stability parameters were calculated according to the models of Eberhart & Russell [7] and Freeman & Perkins [8] using regression coefficient (b_i) and deviation from regression (S^2_{di}), as well as Perkins and Jinks [9] regression coefficient (B_i). Selection index was also performed according to the method of Tallis [10]. In addition, coefficient of variability was estimated according to Francis and Kannenberg [12].

RESULTS AND DISCUSSION

Stability analysis: The combined analysis of variance for three roselle cultivars, Sudani, Masri and White under seven fertilizer treatments was shown in (Table 1). There were highly significant differences between cultivars for all studied characters in both first and second seasons and between environments for all traits, except number of primary branches per plant (NPB) at the second season which failed to reach significant level at 5%. Highly significant variations were also detected for cultivar-environmental interactions of all studied characters in the first season except NPB. Meanwhile, dried sepals yield per plant (DSY) revealed high significant cultivar-environment interaction and significant interactions were detected for NPB and fresh sepals yield per plant (FSY), but non-significant interactions were found in plant height (PH) and number of bolls per plant (NB) in the second season. These results reflect the different responses of roselle cultivars to environmental changes, i.e., seasons and nitrogen fertilizer treatments. Beside that this procedure of statistical analysis was suitable for understanding the cultivar-environmental interaction and stability.

Table 1: Analysis of variance for five characters of three Roselle cultivars under seven nitrogen fertilizer treatments during 2003 and 2004

Source of variance	D.F.	Dried sepals yield/plant (DSY) X_1	Plant height (PH) X_2	Primary branches/ plant (NPB) X_3	Bolls/plant (NB) X_4	Fresh sepals yield/plant (FSY) X_5
				First season		
Cultivars (C)	2	27.85**	7486.50**	15.05**	268.40**	2577.06**
Environments (E)	6	21.05**	274.71**	5.59**	312.74**	856.23**
Replicates in environments	14	3.28	31.44	2.38	45.65	124.25
C × E	12	16.49**	121.22**	4.16	247.64**	680.13**
Error	28	2.05	56.20	3.21	30.03	90.07
				Second season		
Cultivars (C)	2	27.85**	9339.50**	25.84**	397.41**	3252.09**
Environments (E)	6	19.10**	196.17**	1.59	207.43**	861.82**
Replicates in environments	28	0.40	81.55*	3.65	20.82	41.03
C × E	12	3.02**	64.85	8.05**	18.72	115.15*
Error	56	0.70	47.65	4.23	41.94	54.54

Table 2: Pooled analysis of variance for five characters of three Roselle cultivars under seven nitrogen fertilizer treatments during 2003 and 2004, Eberhart & Russell's model

Source of variance	D.F.	Dried sepals yield/plant (DSY)	Plant height (PH)	Primary branches/ plant (NPB)	Bolls/plant (NB)	Fresh sepals yield/plant (FSY)
				First season		
Cultivars (C)	2	9.28**	2495.50**	5.02**	89.46**	859.02**
Environments (E) + C×E linear	18	6.00**	57.47**	1.55	89.78**	246.28**
Environment- linear	1	42.10**	549.55**	11.18**	625.48**	1712.45**
C × E linear	2	9.77**	10.23	0.30	216.60**	319.42**
Pooled deviation	15	3.10**	30.95	1.07	37.16**	138.78**
Sudani cultivar	5	0.82	27.01	0.81	4.67	26.59
Masri cultivar	5	3.72**	52.01**	1.11	48.92**	161.63**
White cultivar	5	4.75**	13.85	1.28	57.88**	228.11**
Pooled error	42	0.68	18.74	1.07	10.01	30.02
				Second season		
Cultivars (C)	2	5.57**	1867.84**	5.17**	79.48**	650.45**
Environments (E) + C×E linear	18	1.68**	21.72*	1.18	16.33	72.81**
Environment - linear	1	22.92**	235.37**	1.91*	248.92**	1034.22**
C × E linear	2	0.05	1.35	3.78**	1.44	3.49
Pooled deviation	15	0.48*	10.20	0.78	2.80	17.95
Sudani cultivar	5	0.58**	10.58	0.48	0.51	23.31*
Masri cultivar	5	0.26	14.58	1.25	4.04	14.95
White cultivar	5	0.59**	5.43	0.62	3.86	15.60
Pooled error	84	0.14	9.49	0.85	8.39	10.91

The pooled analysis of variance described by Eberhart and Russell [7] (Table 2) indicated highly significant differences among roselle cultivars and fertilizer treatment (linear) for all studied characters in both seasons because of the different genetic background of cultivars and considerable variation among environments used in the experiments, which altered the expression of all characters. DSY, FSY and NB revealed highly significant cultivar-environmental (linear) interaction and pooled deviation at the first season. While at the second season, only NPB had high significant difference for cultivar-environmental interaction and significant pooled deviation from regression was detected for DSY. The deviation from regression for each cultivar showed that Masri and White had highly significant deviation for DSY, FSY and NB in the first season, while Sudani and White cultivars had the same deviation for DSY only (Table 2).

The joint regression analysis was conducted for all studied traits according to the procedure described by Perkins and Jinks [9]. All sources of variation mean squares were tested against average error (Table 3). Highly significant differences among cultivars and environments were found for all traits except environments for NPB had significant and non-significant variations in the first and second seasons, respectively and this in turn reveal that this character had less sensitivity for environmental changes. Also, there were high significant differences among cultivar-environmental interaction for all characters except NPB in the first season, while interaction significant differences were detected for all traits except NB in the second season. On the other side, heterogeneity between regression mean squares were highly significant when tested against the remainder mean squares for DSY, FSY and NB in the first season and only for NPB in the second one. However, the remainder mean squares were significant for all characters except NPB when tested against average error at the first season and except NPB and NB in the second season.

The partitioning analysis of variance model of Freeman and Perkins [8] was also conducted for characters under study and illustrated at Table 4. It can be shown that mean squares due to cultivars showed highly significant for PH, significant for DSY, FSY and NPB, but non-significant for NB in the first season. Whereas cultivars mean squares showed highly significant differences for all traits in the second season. Therefore considerable variations among traits expression were detected between roselle cultivars. Moreover, significant variations were noted for PH and DSY at the first season in addition to DSY and FSY at the second one due to environmental changes. The combined regression displayed significant variability for all studied characters except NPB which gave non-significant variance in both seasons. Meanwhile, all remainder items illustrated non-significant values for all studied traits in both seasons.

Table 3: The joint regression analysis of variance for five characters of three Roselle cultivars under seven nitrogen fertilizer treatments during 2003 and 2004, Perkins & Jinks model

Source of variance	D.F.	Dried sepals yield/plant (DSY)	Plant height (PH)	Primary branches/ plant (NPB)	Bolls/plant (NB)	Fresh sepals yield/plant (FSY)
				First season		
Cultivars (C)	2	9.28**	2495.50**	5.02**	89.46**	859.02**
Environments (E) (joint regression)	6	7.02**	91.59**	1.86*	104.25**	285.41**
C × E	12	5.50**	40.40**	1.39	82.55**	226.71**
Heterogeneity between regression	2	9.77**	10.22	0.30	216.60**	319.42**
Remainder	10	4.64**	46.43**	1.60	55.74**	208.17**
Pooled error	42	0.47	12.49	0.71	6.67	20.02
				Second season		
Cultivars (C)	2	5.57**	1867.84**	5.17**	79.48**	650.45**
Environments (E) (joint regression)	6	3.82**	39.23**	0.32	41.49**	172.37**
C × E	12	0.60**	12.97*	1.61*	3.74	23.02*
Heterogeneity between regression	2	0.05	1.37	3.78**	1.44	3.49
Remainder	10	0.71**	15.29*	1.18	4.21	26.93*
Pooled error	84	0.09	6.33	0.56	5.59	7.27

Table 4: Partitioning analysis of variance for five characters of three Roselle cultivars under seven nitrogen fertilizer treatments during 2003 and 2004, Freeman & Perkins model

Source of variance	D.F.	Dried sepals yield/plant (DSY)	Plant height (PH)	Primary branches/ plant (NPB)	Bolls/plant (NB)	Fresh sepals yield/plant (FSY)
				First season		
Cultivars (C)	2	11.17*	5226.56**	16.67*	116.45	1570.75*
Environments (E)	6	10.32*	223.25**	5.94	172.95	517.14
Combined regression	1	41.17*	1298.37**	4.08	556.91**	1720.88**
Residual (1)	5	4.15	8.23	6.31	96.15	276.39
C × E	12	7.72	81.02	4.97	115.90	338.52
Heterogeneity of regression	2	12.78	19.38	12.07	251.49	407.47
Residual (2)	10	6.71	93.35	3.55	88.78	324.73
Error between replicates	21	10.15	655.45	8.58	148.35	558.65
				Second season		
Cultivars (C)	2	6.05**	1836.69**	7.01*	116.23**	702.17**
Environments (E)	6	4.73**	118.45	0.83	50.32	222.78**
Combined regression	1	25.85**	231.34*	0.21	140.04**	1061.51**
Residual (1)	5	0.50	95.87	0.96	32.37	55.03
C × E	12	1.56	19.94	4.10	11.03	51.74
Heterogeneity of regression	2	0.05	8.50	1.56	6.18	10.30
Residual (2)	10	1.86	22.23	4.60	12.00	60.03
Error between replicates	63	70.74	9264.38	79.98	933.67	3234.97

It is evident that all used models of analysis of variance cleared that there were significant genetic background variations between roselle cultivars and the response of tested quantitative characters. Also, significant different changes were displayed due to nitrogen fertilizer treatments (environments). However, all using statistical models confirmed significant cultivar-environmental interaction for most of the studied traits in both seasons. On the other hand, DSY followed by FSY were the most sensitive characters of roselle cultivars in response to environment changes, contradict with NPB which showed more stable trait with different environmental changes. These results are in good agreement with some previous studies [13-14] in roselle cultivars as well as [6, 15, 16] on cotton varieties.

Stability parameters: Five stability parameters: regression coefficient and deviation from regression of Eberhart & Russell [7] model (b_i-ER and S^2_{di}-ER) and Freeman & Perkins [8] model (b_i-FP and S^2_{di}-FP) and regression coefficient of Perkins & Jinks [9] model (B_i-PJ) as well as average values and coefficient of variability (C.V. %) were calculated for each of the studied characters of roselle cultivars among both the first and second seasons and illustrated at Table 5. Up normally values for b_i-FP and S^2_{di}-FP were detected for all characters and did not

Table 5: Stability parameters estimated for DSY, PH and NPB of three Roselle cultivars grown under seven nitrogen fertilizer treatments during 2003 and 2004

Characters	Stability parameters	First season			Second season		
		Sudani	Masri	White	Sudani	Masri	White
Dried sepals yield /plant (DSY)	$\bar{x}$	12.43	14.73	13.69	13.47	15.25	14.25
	$b_{i\,E.R.}$	0.71	0.35	1.94	1.07	0.92	1.01
	$S^2d_{i\,E.R.}$	0.82	4.75	3.72	0.58	0.59	0.26
	$B_{i\,P.J.}$	-0.29	-0.65	0.94	0.07	-0.09	0.01
	$b_{i\,F.P.}$	0.34	0.11	1.02	0.20	0.18	0.18
	$S^2d_{i\,F.P.}$	-8.32	-4.06	-3.44	-70.40	-70.38	-70.39
	C.V.%	10.96	13.99	25.22	10.34	8.17	8.68
Plant height (PH)	$\bar{x}$	146.14	164.86	183.90	149.37	164.89	182.03
	$b_{i\,E.R.}$	1.19	1.08	0.74	0.89	1.15	0.97
	$S^2d_{i\,E.R.}$	27.01	13.85	52.01	10.58	5.43	14.58
	$B_{i\,P.J.}$	0.19	0.08	-0.27	-0.11	0.15	-0.03
	$b_{i\,F.P.}$	1.28	1.67	1.12	0.15	0.30	0.25
	$S^2d_{i\,F.P.}$	-617.72	-612.63	-573.56	-9251.28	-9252.67	-9254.12
	C.V.%	5.54	4.16	4.20	2.93	2.82	2.71
No. of primary branches/plant (NPB)	$\bar{x}$	14.05	14.43	15.67	14.06	14.31	15.66
	$b_{i\,E.R.}$	1.32	0.77	0.92	1.04	-1.46	3.42
	$S^2d_{i\,E.R.}$	0.81	1.28	1.11	0.48	0.62	1.25
	$B_{i\,P.J.}$	0.32	-0.23	-0.09	0.04	-2.46	2.42
	$b_{i\,F.P.}$	1.45	0.89	-1.03	0.08	-0.24	0.05
	$S^2d_{i\,F.P.}$	-5.60	-5.02	-3.93	-79.30	-79.38	-78.71
	C.V.%	9.43	8.30	7.68	5.11	6.03	9.64
No. of bolls/plant (NB)	$\bar{x}$	44.14	46.38	51.19	48.57	53.40	55.06
	$b_{i\,E.R.}$	0.65	0.20	2.15	1.11	0.86	1.03
	$S^2d_{i\,E.R.}$	4.67	57.88	48.92	0.51	3.86	4.04
	$B_{i\,P.J.}$	-0.35	-0.80	1.15	0.11	-0.15	0.03
	$b_{i\,F.P.}$	0.26	0.06	1.11	0.13	0.07	0.15
	$S^2d_{i\,F.P.}$	-120.60	-77.72	-18.64	-927.47	-931.19	-928.27
	C.V.%	9.81	15.19	27.65	8.63	6.84	7.73
Fresh sepals yield/plant (FSY)	$\bar{x}$	85.85	103.80	83.57	93.23	107.61	89.30
	$b_{i\,E.R.}$	0.76	0.40	1.84	1.09	0.89	1.02
	$S^2d_{i\,E.R.}$	26.59	228.11	161.63	23.31	15.60	14.95
	$B_{i\,P.J.}$	-0.24	-0.60	0.84	0.09	-0.11	0.02
	$b_{i\,F.P.}$	0.46	0.13	1.04	0.21	0.18	0.15
	$S^2d_{i\,F.P.}$	-474.17	-213.40	-216.85	-3222.41	-3220.11	-3218.62
	C.V.%	10.21	13.81	25.57	10.06	7.12	9.49

correspond with other stability parameters, so they had not any consideration in the following discussion.

Dried sepals yield per plant (DSY): Data presented at Table 5 cleared that Sudani could be considered as a stable cultivar for the trait of DSY in the first season, due to it had the nearest b_i-ER to the unity (0.71) and had minimum value of S^2_{di}-ER (0.82) approached to zero. Sudani had also the lowest B_i-PJ value (-0.29) and C.V. % value (10.96 %) to confirm the stability for DSY. On the contrary, White cultivar had b_i-ER value (1.01) not significantly different from 1.0, S^2_{di}-ER (0.26) not significantly different from zero, approaching B_i-PJ value to zero (0.01) and low value of C.V. % (8.68%) to appear its stability for DSY in the second season.

Plant height (PH): All cultivars exhibited up normal deviation values from regression S^2_{di}-ER for PH, but Masri cultivar exhibited 1.08 as a coefficient of regression b_i-ER not significantly from unity and minimum B_i-PJ (0.08) nearest to zero, as well as lowest C.V. % (4.16) and thus appear to be most stable cultivar for PH in the first season. Meanwhile, approaching b_i-ER value to 1.0 and minimum S^2_{di}-ER addition to nearest B_i-PJ value to zero

Table 6: Ascending order of seven nitrogen fertilizer treatments based on selection index of the characters of three Roselle cultivars during 2003 and 2004

Seasons	Sudani	Masri	White
First season	t_1	t_4	t_2
	t_5	t_1	t_1
	t_3	t_6	t_5
	t_7	t_3	t_6
	t_6	t_7	t_7
	t_2	t_2	t_4
	t_4	t_5	t_3
Second season	t_1	t_1	t_1
	t_6	t_2	t_6
	t_2	t_5	t_2
	t_4	t_6	t_5
	t_5	t_7	t_7
	t_7	t_3	t_3
	t_3	t_4	t_4

and lowest C.V. % were found to have for PH of White cultivar to reveal stable property in the second season (Table 5).

Number of primary branches per plant (NPB): NPB had no significantly positive values of b_i-ER and B_i PJ addition to lowest C.V. % value for White cultivar in the first season.While same observations were found for Sudani cultivar in the second season. Thus White and Sudani cultivars seemed stable properties for NPB in both seasons, respectively.

Number of bolls per plant (NB): Data presented at Table 5 showed that the nearest values of b_i-ER to unity, B_i-PJ to zero and low C.V. % value for NB were found to be for Sudani cultivar in the first season and White cultivar in the second season indicating stability of these cultivars to NB trait.

Fresh sepals yield per plant (FSY): Data in Table 5 revealed that Sudani cultivar in the first season and White cultivar in the second season had the prefer values of stability parameters. Therefore they presented the same stability properties for NB to FSY.

Generally, the regression coefficient values computed from the three stability models showed wide range for all studied characters suggesting that the cultivars responded differently to the environmental changes (nitrogen fertilizer treatments or seasons). Sudani cultivar was stable for DSY, NB and FSY, Masri cultivar was stable for PH and White cultivar was stable for NPB in the first season. Meanwhile, White cultivar was stable for all characters except NPB found to stable for Sudani cultivar in the second season. However, previous studies [13, 14] in roselle cultivars as well as [6, 17] in cotton varieties have shown genotypes often demonstrate desirable by stability parameter estimates.

Selection index: According to selection index of all five characters expression for roselle cultivars, under all tested environments (seven nitrogen fertilizer treatments) were arranged in ascending throughout the two investigated seasons are recorded in Table 6. Results of the first season showed that the maximum response of selection index was t_4, t_5 and t_3, while the minimum response was t_1, t_4 and t_2 for Sudani, Masri and White roselle cultivars, respectively. In the second season, maximum selection index was achieved with t_3, t_4 and t_4, while minimum values were obtained with t_1 for all tested roselle cultivars i.e., Sudani, Masri and White. The results indicated also that all other responded selection index were in between values. These results showed that arrangement of nitrogen fertilizer treatments differed from cultivar to another and from season to another and this in turn shows the different responses of different cultivars and environments. On the other hand, chicken manure (t_4) took the first rank for Sudani in the first season and for Masri and White for the second. Also, 200 kg ammonium sulfate /feddan (t_3) was the best environment for White and Sudani cultivars in the first and second seasons, respectively. Therefore treatments of 20 m^3 chicken manure/feddan followed by 200 kg ammonium sulfate/feddan were the best preferred nitrogen fertilizers and thus they have must recommended for roselle plants in such environmental conditions.

The obtained values of the five studied characters for each roselle cultivar responded by the preferred nitrogen fertilizer treatment among two seasons were illustrated in Table 7. It is cleared that Masri under t_5 was the dominant cultivar for DSY and FSY compared with other cultivars in both seasons. While White cultivar under t_3 had the maximum values of PH and NB in both seasons too. Concerning to the trait NPB, Sudani and White cultivars had the same highest value (14.33) under t_4 and t_3, respectively in the first season, while the highest NPB (15.60) was found in Masri cultivar of the second season. On the other hand White cultivar produced the higher values for all traits in the first season comparing with those in the second one. Meanwhile, Masri had the maximum values for DSY, FSY and NB in the first season, but maximum PH and NPB in the second one. Contradict with Sudani cultivar produced the maximum DSY, FSY and

Table 7: Values of five quantitative characters for three Roselle cultivars under the best nitrogen fertilizer treatments among two seasons

Seasons	Cultivars	The best nitrogen fertilizer treatment	Dried sepals yield/plant (g) (DSY)	Plant height (cm) (PH)	No. of primary branches/plant (NPB)	No. of bolls/plant (NB)	Fresh sepals yield/plant (g) (FSY)
First season	Sudani	t_4	14.20	155.00	14.33	49.00	94.63
	Masri	t_5	19.00	159.33	13.67	61.00	133.63
	White	t_3	18.87	191.33	14.33	72.67	115.67
Second season	Sudani	t_3	14.50	146.20	14.00	52.80	102.90
	Masri	t_4	16.90	168.00	15.60	55.00	116.60
	White	t_4	16.26	176.80	13.80	60.20	103.58

NB in the second season and maximum PH and NPB in the first one. These results showed that the cultivars affected differently by different environmental changes and thus their character expressions responded differently too. It could be concluded that similar results were obtained [4-6, 17] reported that the genotypes under each study influenced differently under different environmental conditions and these in turn confirm our findings.

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