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Investigating the Effect of Heat Shock on Chromosomal Aberration of Vicia dasycarpa

Sanaz FAREGHİ[1,*], Varahram RASHİDİ[2], Ahmad RAZBAN HAGHİGHİ[3]

[1]*M.A Student in Agricultural Engineering, Department of Plant Breeding (Biometry), Tabriz Branch, Islamic Azad University, Tabriz, İran*

[2]*Assisstant Professor, Tabriz Branch, Islamic Azad University, Tabriz, İran*

[3]*Trainer, Faculty member in Natural Resources and Agricultural Researches Center of Khosroshahr, East Azerbaijan Province*

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Abstract. One of the wide applications of cytogenetic is evaluation of environmental factors on chromosomes. In this study, some seeds were boiled in bath water of 900 C for 3 minutes in order to study effects of heat shock on chromosomal aberration of hairy vetch. Then root-tip meristems of both kinds of seeds (i.e. normal seeds and seeds obtained under heat shock conditions) were studied from metaphase mitosis aspect. Normally plant is diploid with basic chromosome number x=7, (2n=2x=14). Cytogenetic studies of the seeds under heat shock conditions indicated mixoploidy state, in a way that diploid, aneuploid and tetraploid cells were observed in a root. Results of variance analysis and comparison of average normal treatments with heat shock treatments in diploid state indicated increased length of chromosomes and increased amount of genome. No aberration of chromosomal size was observed in tetraploid cells compared to normal state, just number of chromosomes increased twofold (2n=4X=28). Moreover study of some morphological traits indicated significant difference in the plant height of heat shock conditions compared to normal plants.

Key words: meristem, mixoploidy, aneuploid, metaphase, genome

1. INTRODUCTION

Rangeland ecosystem destruction and irregular exploitation of meadows result in country's soil and water resources destruction. This makes the necessity of paying attention to forage plants cultivation more important. Mashak known as Vetch [1] in English, is one of forage plants which has been cultivated in different types since past [2]. Vetch, with scientific name *Vicia dasycarpa*, is from vicia genus and Legominosase family. Most plants of genus are diploid with basic chromosom number X=5,6,7,(2n=2x=10-12-14). Though, tetraploid and hexaploid have been also reported in this genus [3]. Considering the importance of forage plants especially plants of Legominosae family, in animal feed, nitrogen saving, providing soil fertility and intercropping with cereals, identification of vetch forage plant and developing it's cultivation is of great importance [4]. One application of cytogenetic is evaluation of environmental factors on chromosomes and finally people's health [5]. Among the environmental factors influencing chromosomes, following factors have been reported till now: higher temperatures and herbicides [6], lack of water [7], lack of nutritive elements and soil salinity [8], ray [9], chemical factors such as fungicides [10]. Plants, usually, confront heat stress at temperatures higher than 35-45^0 C and cold stress at temperature higher than 0^0C at temperature domain of 0-15 [11]. Tissues which are growing actively, survive at heat higher than 45^0C rarely, this is while dry seeds can tolerate temperature of 120^0C and pollens can

* *Corresponding author. Email address: S1978_Fareghi@yahoo.com*

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tolerate temperature of 75^0 C [12]. Applying heat shock of 45^0 C, on *Vicia faba* seeds (a diploid plant of Legominosae with chromosome number 2n=2x=12) for 1 and 2 hours, and root meristem metaphase cells study indicated breaks, fragments and pulverization of chromosomes and also chromosomes number increased twofold (i.e. cell with chromosome number of 2n=4x=24) [13]. Moreover applying heat shock of 55^0 C on *Lathyrus sativus* seeds (a plant from Legominosae) for 24, 48 and 72 hours and studying miosis division of M2 plant flower bud indicated cytomixis and multivalent formation in metaphase, chromosomes breaks due to translocation and inversion. Moreover a significant aberration in flower stain was observed in plants under heat shock compared to control group [14].

Normally, durum wheat is tetraploid (2n=4x=28). Applying temperature stress of 35^0 C to the seeds and root meristem cells metaphase study, indicated abnormal cytokinesis and increased multiploid cells with ploid level of (8x=56), (16x=12) [15]. Moreover as a result of corn seed cells treatment (at initial stages of division) with temperature of 38-40 ^{0} C, tetraploid stems were obtained and also with putting wheatear at early stage of seed division at temperature of 23-25^0 C for 20 hours and then placing them at temperature of 43^0 C for 20-30 minutes were succeeded to produce polyploid plants[16]. The aim of present study is to investigate the effects of heat shock on chromosomal aberrations of hairy vetch with scientific name of *Vicia dasycarpa*.

2. MATERIALS AND METHODS

Present study was carried out during 2013-2014 academic year in genetic laboratory of department of natural resources, natural resources and agricultural researches center of Khosroshahr, East Azerbaijan. First seeds were divided into two groups of normal seeds and heat shock seeds to study the effects of heat shock on chromosomal aberrations of hairy vetch (a plant from Vicia genus and Legominosae family). Then normal and heat shock seeds were cultivated separately in petri dishes between two layers of humid filter paper. For heat shock induction, seeds were boiled in bath water of 90 ^{0} C for 3 minutes. After budding, when radicles grew 7mm to 10 mm, pretreatment was performed using 0.05% colchicine at room temperature for 2 hours then they were fixed in farmer solution (i.e. glacial acetic acid and alcohol(1:3)) inside refrigerator at 4^0 C for 24 hours. After staining with 2 % acteo orcein solution inside refrigerator at 4^0 C for 11-12 hours they were ready to be studied. Slides were prepared in squash method (with 45% acetic acid squash solution). After providing appropriate metaphase cells photo, karyotype was prepared for each normal and under shock treatment at diploid and tetraploid state using Adobe Photoshop Cs_5 software. After preparing 5 karyotypes for each treatment, total length of chromosomes (L+S), long arm length (L), short arm length, arms ratio (L/S) and centromeric index (S/L+S) and satellite length were calculated using Micro measure software . Stebbines tow-way table[17] was used to determine karyotype symmetry state and chromosomes difference of range of relative length index (DRL) along with total karyotype form percentage (TF %) were calculated also. Levan and Sandberg method was used to classify chromosomes and to determine cenromer place.

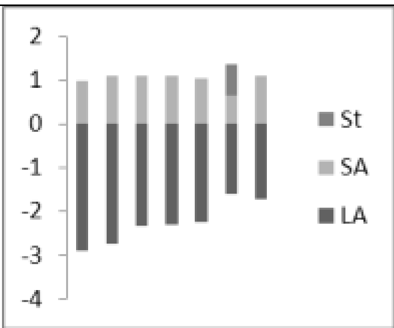
In addition to cytogenetic studies, morphological studies were also done with cultivation of 3 seeds of each treatment in 12 pots for each normal and under heat shock treatments and also through investigation of characteristics including height, number of leaves and number of stem nodes, 10 days after plant growth. Variance analysis was carried out in complete randomized design with 5 repetitions to study the effects of heat shock on karyotypic and morphological characteristics. T-test was used to compare averages between normal treatment and heat shock treatment in diploid and tetraploid states. XIstata SPSS was used for statistical analysis and also Excel was used to draw ideograms and some calculations.

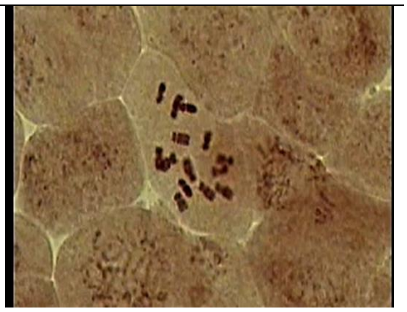
3. RESULTS AND DISCUSSION

Mixoploid state was observed under heat shock conditions of metaphase studies. In other words diploid and tetraploid cells were observed in one root and in some other roots aneuploid cells with 13 or 15 chromosomes were observed accordingly. Studies indicated that under normal conditions, the plant is diploid with basic chromosome number x=7(2n=2x=14). Photos of normal treatment mitosis metaphase and treatments under shock in diploid and tetraploid states along with karyotype and ideogram can be seen in Fig 1, and results obtained from karyotype analysis have been displayed in table 1.

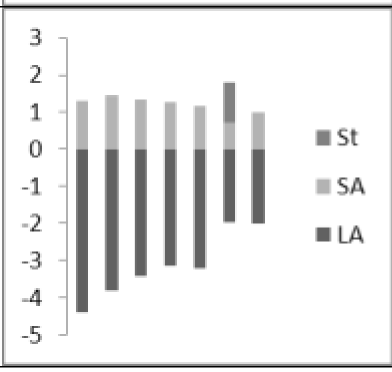
According to Fig.1and Table 1, normal treatment with 14 chromosomes, and satellite in short arm of chromosome pair 6 and diploid heat shock treatment with14 chromosomes and satellite in short arm of chromosome pair 6 and tetraploid heat shock treatment with 28 chromosomes and satellite in short arms of chromosome pairs 11 and 12 can be seen evidently. According to Stebbines classification, normal and tetraploid heat shock treatments are at class 3A, but diploid heat shock treatment is in class 4A. Karyotypic formula is in normal treatment 6(sm)+1(m), diploid shock treatment 6(sm)+1(st) and tetraploid shock 14(sm).

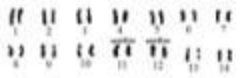
***v. dasycarpa*(2n=2x=14)- Normal**





***v. dasycarpa*(2n =2x=14) - Heat shock**





***v. dasycarpa*(2n =4x=28)**
- Heat shock

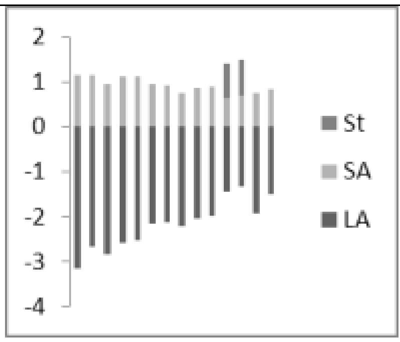


Fig. 1. Metaphase cells, karyotype and ideogram of studied treatments.

Table1. Karyotypic characteristics of normal and heat shock treatments at diploid and tetraploid states. G: Genome (total length of hapoloid chromosomes), L: Long arm length, S: Short arm length, L+S: Total length of chromosome, AR: Chromosomal arms ratio, CI: Centromeric index, St: Stebbines symmetry class, DRL: Biggest and smallest chromosomes difference of range of relative length, TF%: Total karyotype form percentage and KF: Karyotypic formula.

Treatment	2n	G	L+S	L	S	CI	AR	%TF	DRL	KF	St
Normal	14	23.6±0.21	3.37±0.03	2.26±0.02	1±0.02	0.3±0.003	2.36±0.05	29.74	4.47	6 (sm)+1(m)	3A
Heat shock-diploid	14	31.35±2.92	4.48±0.42	3.15±0.31	1.18±0.1	0.27±0.008	2.78±0.09	26.42	8.52	6 (sm)+1(st)	4A
Heat shock-tetraploid	28	44.89±2.21	3.21±0.16	2.19±0.08	0.91±0.06	0.28±0.005	2.42±0.09	28.5	4.44	14(sm)	3A

After Bartlet test and making sure of reliability of assumptions in anova such as homogeneity of trial errors variance, traits variance analysis were carried out.

Complete randomized design was used in both states to compare measured cytogenetic traits between normal treatment and heat shock treatments. Results of this experiment which was 2 treatments with 5 repetitions, can be seen in table 2. Results of t-test for average comparison of significant traits have been presented in table 3.

Table2. Mean square of studied karyotypic characteristics between normal treatment and diploid heat shock based on CRD design.

Aberration sources	Degree of freedom	Mean Square						
		Genome	Satellite	Centromeric index	Arms ratio	Short arm	Long arm	Total length of Chr.
Between treatments	1	149.9238*	0.29584	0.00289* *	0.44944**	0.07921	1.94481*	3.04704*
Trial error	8	21.38225	0.097	0.00019	0.026815	0.024325	0.236705	0.4364
CV%	-	16.83	35.23	4.87	6.37	14.27	17.98	16.83
Mean	-	27.474	0.884	0.283	2.568	1.093	2.705	3.924

*and** are significant at probability level of 5% and 1% respectively.

Table3. Results of comparison between traits mean related to chromosomal size of normal and diploid heat shock treatments.

t/Treatment	Total length of Chr.	Long arm	Arms ratio	Centromeric index	Genome
Normal	3.372	2.264	2.356	0.3	23.602
Heat shock-diploid	4.476	3.146	2.78	0.266	31.346
t	2.6476*	2.8664*	4.0939* *	3.90007* *	2.6479*

*and** are significant at probability level of 5% and 1% respectively.

These findings show that normal treatment and treatment under shock (diploid) have significant difference at level of significance of 1% for traits (arms ratio and centroemric index) and they have significant difference at level of significance of 5% for traits (total length of chromosome, long arm and genome). Genetic diversity for characteristics related to genome and chromosome size are considered for trait selection. Although they didn't indicat differences in chromosome number, difference in most chromosomal characteristics indicates increasing length of chromosomes.

Moreover regarding normal treatment and tetraploid treatment mean comparison was carried out with t-test to compare cytogenetic traits which indicated no significant difference in non of traits except for genome. This is due to duplication of number of chromosomes. Results of comparison can be seen in table 4.

Table4. Results of comparison between traits mean related to chromosomal size of normal and tetraploid heat shock treatments.

t/Treatment	Total length of Chr.	Long arm	Short arm	Arms ratio	Centromeric index	Genome
Normal	3.372	2.264	1.004	0.712	0.3	23.602
Heat shock-tetraploid	3.21	2.19	2.425	0.775	0.285	44.895
t	1.61	1.253	0.727	0.368	2.535	17.402**

** significant at probability level of 1%.

Moreover, in this research, for investigation of morphological effects of heat shock, some morphological traits such as: height, number of leaves and number of stem nodes to compare normal treatment and heat shock treatment were studied. Results indicated significant difference at probability level of 5% in characteristic of height of normal treatment and heat shock treatment. Results of traits measurement, results of variance analysis with CRD design and comparison of mean with T-test can be seen in tables 5,6 and 7 respectively.

Table5. Results of measuring morphological traits of normal treatment and treatment under heat shock.

Treatment	Height	Number of leaves	Number of nodes
Normal	1.6±16	1.4±15.4	0.41 ±4.2
Heat shock	1.07±22.16	2.2±19.34	0.4±4.9

Table6. Mean square of morphological traits studies with normal treatment and heat shock treatment based on CRD design.

Aberration sources	Degree of freedom	Mean Square		
		Height	Number of leaves	Number of nodes
Between treatments	1	94.864*	38.809	1.225
Trial error	8	9.314	176.0515	0.8125
CV%	-	16	23.77	19.81
Mean	-	19.08	17.37	4.55

* significant at probability level of 5%.

Table7. Result of comparison of height mean between normal treatment and heat shock treatment.

t/ Treatment	Height
Normal	16
Heat shock	22.16
t	3.914*

* significant at probability level of 5%.

According to Rui-Ju et al (2006), Kumar and Tripathi (2009), reliability of these results is based on the effects of heat shock on faba bean plant and grass pea on creating mixoploid state in cells and producing tetraploid cells compared to normal diploid cells and morphological effects of heat shock in flower stain and advent of significance difference in height, width and length of the leaf.

4. CONCLUSION

1. Results obtained from present study indicated that *Vicia dasycarpa* enjoys ploid-diploid level under normal conditions and it's basic chromosome number is x=7(2n=2x=14).
2. Findings indicated that following 90^0 C heat shock for 3 minutes to seeds of the plant, mixoploid cells involving diploid and tetraploid and rarely aneuploid are produced.
3. Variance analysis results and mean comparison indicated significant difference in most karyotypic traits including total length of chromosome, long arm, genom, arms ratio and centromeric index between normal treatment and heat shock treatment in diploid state. Inspite of equal number of chromosomes, significant difference of genome is caused by increase in chromosomes length due to heat shock. This is while when comparing normal treatment and tetraploid heat shock treatment no significant difference was observed in non of karyotypic parameters, except for genome increase which is related to duplication of number of chromosomes.
4. According to karyotypic formula, shock treatment at diploid state with formula 6(sm)+1(st) compared to normal treatment with formula 6(sm)+1(m) and tetraploid heat shock treatment with formula 14 (sm) have less karotypic symmetry. Results of TF% and Stebbines confirm the issue also.
5. Morphological studies of the heat shock and normal treatment indicate significant difference at plant height under heat shock treatment compared to normal plants.

REFERENCES

[1] Mozaffariyan,V. (2007), "Dictionary of Iranian Plants Names", Amir Kabir Press, (in Persian).
[2] Karimi,H.(2000), "Forage Plants Breeding and Cultivation", University of Tehran Press, (in Persian).
[3] Gaffarzadeh Namazi, L., Badarzadeh, M. and Asghari Zakaria, R. (2008), "Karyotype of several vicia species from iran", Asian J. of Plant Sciences, 7(4), pp. 417-420.
[4] Mazaheri Laghab, H., Aram, Y., Chaichi, M and Akbari, GH.(2009), "An Introduction to Forage Plants" Aboo Ali Sina University Press, (in Persian).
[5] Feheimer, N .S. (1979), "Cytogenctic in Plant Production", J. of Dairy Science, 62(5).
[6] Bajpai, A. and Singh, A.K. (2006), "Meiotic behavior of Caria Papaya: spontaneous chromosome instability and elimination in important cvs in north Indian Conditions", Cytologia, 71, pp. 131-136.
[7] Saini, H. S. (1997), "Effect of water stress on male gametophyte development in plant sex", Plant Reproduce, 10(6), pp. 67-73.
[8] Sun, K., Hunt, k . and Hauser, B.A. (2004), "Orule abortion in Arabidopsis triggered by stress", Plant Physiol, 135, pp. 2358-2367.
[9] Vicini, L. F. and Carvalho, C. R. (2002), "Meiotic chromosomal variation. resulting from irradiation of pollen in maize", J. Appl. Genet, 43, pp. 463-469
[10] Fairbanks, M.M., Hardy, G.E. and Mc Comb, J.A. (2002), "Mitosis and Miosis in plants are effected by the fungicide phosphate. Aust", Plant Pathol, 31, pp. 281-289.
[11] Mir Mohammadi Meybodi, S.A.M and Tarkesh Isfahani,S. (2004), " Racial and physiological aspects of cold stress and freezing in Forage Plants" Golbon Press, (in Persian).

[12] Haghani,S and Ebrahimi, M.(2009), "temperature stresses", www.agrono millennium . com /pdf/tanesh damaee 2.pdf, (in Persian).
[13] Rui- Ju, L., Qiao - Ling, Q., Yue-fang,S., Run- tain, H., She- teng, K., Ling - bo, X .and Jian- hue, H. (2006), "Response of in vitro propagated plants of vicia faba to high temperature and alter violet stress", J. of Nuclear Agri- Sci, 20(6), pp. 469-472.
[14] Kumar, G. and Tripathi, R. (2009), "Influence of heat stress on genome of grass pea", J. of Environmental Biology, 30(3), pp. 405- 408 .
[15] Klindworth, D.L . and Williams, N.D. (2001), "characterization of a mitotic mutant of durum wheat", Chromosome Research, 9,pp. 377-386.
[16] Farsi, M and Bagheri, A. (1999), "Principles of plant breeding", Jahad Daneshgahi of Mashhad Press, (in Persian).
[17] Stebbins, G. L. (1971), "Chromosomal evolution in higher plants", Edward Arnold Publisher LDT, London.