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Correlation between genetic diversity and genotype of advanced atmospheric weather conditions Ahvaz

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***Abstract*—**In order to determine the genetic diversity and heritability promising genotypes of barley crops in 2012-2013 form of randomized complete block design with three replications was conducted at the Agricultural Research Station in Ahvaz. Plant height, peduncle length, spike length, awn, awn length, spike-free, grain weight, flag leaf area, number of spikelets per spike, harvest index, number of spikes per square meter, number of seeds per square meter, number of seeds per spike, yield, biological yield, days to maturity, days to anthesis, days to flowering, grain filling duration was measured**.** The results of the analysis of variance showed significant differences among genotypes for allCharacters studied.there was an indicate that genetic diversity in the genotypes. 19,3,15,5,2genotypeshadthe highest grain yield.Correlation coefficients showed the highest correlation attribute of grains per spike ($r=0.96^{**}$) with grain yield. Principal components analysis of 17 variables into six components, announced that the six components of (87.6%) of the variation explaineGay traits days to come, days to anthesis, days to flowering, number of grains per square meter for the first component and the harvest index, number of kernels per ear, yield, biological yield for the second component of the main characters. Results of cluster analysis, the genotypes were evaluated in three different groups. Cluster 1 consists of six genotypes of four character figures that were higher than average. Cluster 3 to 6 trait were higher than average. Cluster 2 with 8 trait were higher than average and some intermediate traits.

***Keywords*—**cluster analysis, correlation, Genetic diversity ofbarley,principalcomponents analysis,.

INTRODUCTION

Genetic diversity is the basis of the natural evolution of plant breeding and biological stability of the system is a critical component [2]. Assessment of genetic diversity in crop breeding programs andconservation reserves is critical to use inheritance.Awareness of genetic diversity in plant species selection races for p Ensure appropriate and predictable hybrid vigor hybrid hybrid products that are especially important to have commercial value arentsDiversity and choice of two main pillars of the reform program and select the desired subject to variation as to the purpose of the study is Change is to take advantage of the diversity and new germplasm resources assessment seems necessary [7].barley, one of the oldest and most valuable satiety and cold cereal plants on land is under cultivation, with nearly (2 m/h), the second-largest corn crop in the country. Much of what is needed in the human food supply comes directly or indirectly from the atmosphere [9]. How much does it cost assessment of the genetic material But the need for better use of genetic resources germplasm leading to better understanding of the efficiency of these resources and allowing them to provide It is necessary. In this regard, given the large number of plant germplasm and the need to be aware of the nature of the relationships between variables,Classification of figures based on the traits and ultimately determine the degree of similarity and genetic distance between them using, Multivariate statistical methods such as principal component analysis and cluster analysis seems necessary [4].This study aimed to determine the correlation of genetic diversity and the atmosphere was inspiring.

Materials andMethods

The program crops in Ahwaz Agricultural Research Center 2012-2013 in a randomized complete block design with three replications was conducted on 20 barley lines. Eachplot wasplantedwith a spacingof (20cm)line6on twostacksof6 metersand the area ofeach plot(7.2m)was Afterremoving(0.5mm)topand bottomof each plot, harvestedareaofeach plotwasplanted(6m). Planting, harvesting desirablyperformedduring the growing seasonandafter harvestplant height,peduncle length,spikelength,awn,withoutawnlength, grain weight,flag leaf area, number of spikeletsper spike, harvest index, number of spikes persquare meter, number of seeds persquare meter, number of kernelsper ear, yield,biological yield,days to maturity, days to anthesis, days to flowering, grain fillingdurationdatawere taken. Data analysis in a randomized complete block design using the software Minitab 16, MSTATC, EXCEL was.Thecorrelation coefficientsbetweentraitsand cluster analysiswere studied

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andthenprincipalcomponent analysiswas performedusingMinitab 16software.

Results and Discussion

Grain yield was significantly correlated with the number of grains per spike ($r=0.96^{**}$) (Table 3). Neyestani and colleagues (2005) reported that the correlation of these traits with grain yield estimated ($r=0.68^{**}$)is the number of grains per spike and grain yield components of large number of grains per spike increases the performance of [10]. Eskandari and Clear Intentions (1999) stated in his research that increases with the increase in the number of spikes per square meter Besides increasing the number of grains per spike, which led to higher number of grains per square meter are positive and significant effect on the performance increase is consistent [2]. Yield significantly positively correlated with the number of grains per square meter($r=0.94^{**}$). In principal component analysis eigenvalues greater than 1 for components 1 to 6 are respectively 4.28, 20.1, 14.8, 1.6, 7.3, 6.5 and (87.6%) of the total variance explained (table 1). Given the importance of the first two components, the first component of the eigenvectors relative values of the coefficients showed that number of seeds per square meter, number of days to maturity, days to anthesis, days to flowering, the second component of spike m square of the number of grains per ear, yield, biological yield were the most important attributes for grouping genotypes. Farahani and cheapness (2009) reported that the four components of the traits days to (50%) heading, days to (50%) pollination, days to maturity, plant height, spike length had the largest share of the explained variation [3]. The second component of grain yield, harvest index, grain weight per spike, number of grains per panicle (Table 1).[5], Hmza and colleagues (2004) examined the diversity among Tunisian winter barley cultivars to 26 of 12 agronomic traits were measured and analyzed using principal component analysis and on the analysis of cluster genotypes traits, and did. ThePrincipal component analysis led to the identification of the first four components which were (87%) of total variation in became. Grouping the results based on principal component analysis and cluster analysis were consistent with each other. The results of cluster analysis indicated that genotypes were divided into 3 groups (Fig 2) Cluster 1 with 6 genotypes included the data in terms of harvest index, leaf area, peduncle length, plant height was higher than average. Cluster 2 with 8 genotypes containing the figures of characters without awn length, number of spikelets per spike, number of spikes per square meter, number of seeds per square meter, number of kernels per ear, yield, biological yield, days to maturity, days to anthesis, days to flowering and higher than average peduncle length, grain weight, flag leaf area, harvest index values are intermediate. Cluster 3 with 6 genotypes included the data in terms of grain weight, grain filling duration, spike length and awn higher than average in terms of plant height, peduncle length, flag leaf area, number of grains per spike, the biological yield, days to maturity, days to anthesis, days to flowering were lower than average (table 2). Genotype distribution diagram based on two factors, first and second suggests that genotypes are in the situation (Fig 1).

Results

Given sufficient variation and selection for improved agronomic traits desired results can be expressed by taking Thegenotypesincluster2has the best performance characteristics are And for the yield increase in the atmosphere is the most important breeding purposes. They can be used in breeding programs. Between genotype and examined the factors genotype (19) having the highest number of seeds perspike with spikeletsper spike, spike length and awnandawn able to produce the highest yield among all genotypes. Based on 19 genotypes with pedigreegen otype(VIOLETA / MJA / / CM67), which is part ofthe newgenotypestestedin the regionwithgoodyieldsin mostofthe studiedtraitsassuperiorgenotypeis selected.

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Table.1eigenvalues,percentageof variance andeigenvectorscoefficientsoftraits inthe principalcomponents analysis.

Component 6	Component 5	Component 4	Component 3	Component 2	Component 1	Adjective
-0.26	-0.21	0.41	-0.27	-0.12	-0.06	PH
0.13	-0.18	-0.56	-0.04	0.13	0.00	PL
-0.45	0.54	-0.34	0.11	-0.11	-0.02	LSA
0.32	0.44	0.07	-0.15	-0.34	-0.15	LS
-0.39	-0.15	-0.28	0.15	-0.11	-0.28	WTG
-0.12	0.37	0.22	-0.38	-0.17	-0.11	FLA
0.35	0.20	-0.29	0.07	-0.24	-0.06	NSS
0.00	-0.12	-0.26	-0.55	0.08	-0.04	HI
-0.06	-0.11	0.05	0.33	-0.36	0.19	NSSM
0.21	-0.16	0.00	-0.21	-0.16	0.35	NGM
-0.00	-0.11	-0.22	-0.20	-0.39	0.18	NSM
-0.07	-0.20	-0.14	-0.20	-0.41	0.18	Y
-0.08	-0.10	0.13	0.32	-0.37	0.19	BY
0.10	0.18	0.00	0.05	0.16	0.41	DM
-0.15	0.15	-0.02	-0.05	0.15	0.41	DF
0.10	0.18	0.00	0.05	0.16	0.41	DP
0.44	-0.07	0.06	0.19	-0.09	-0.30	PG

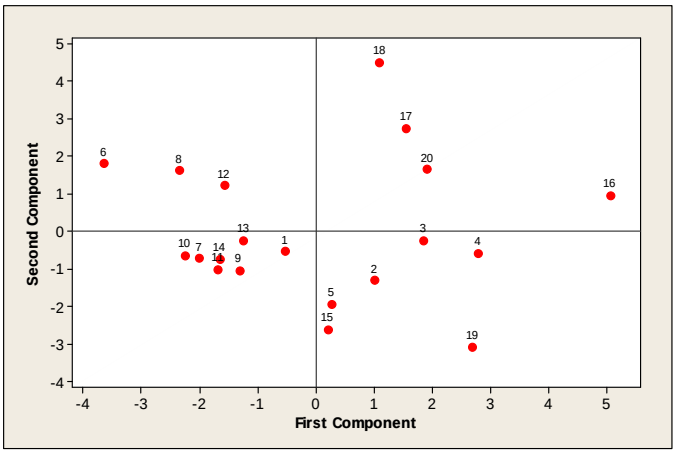

Fig. 1scatterdiagram of20barley genotypesbased onthe first component,thesecond component

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Table.2number ofclusters, the number of genotype, ANOVA and mean comparisonbetweenclusters

Cluster3 (n=6)	Cluster2 (n=8)	Cluster1 (n=6)	Mea Square	Adjective
89.58 a	93.44 a	96.83 a	78.87^{ns}	PH
25.26 a	24.52 a	24.68 a	0.98^{ns}	PL
15.58 a	14.48 a	14.76 a	1.27^{ns}	LSA
4.45 a	4.78 a	4.66a	0.40^{ns}	LS
50.44 a	47.87 a	48.22 a	12.50^{ns}	WTG
4.02 a	4.15 a	4.66a	0.70^{ns}	FLA
14.60 a	15.21 a	14.02 a	2.42^{ns}	NSS
35.53a	35.55 a	37.60 a	8.87^{ns}	HI
362.48 b	529.35 a	364.58 b	6599.30*	NSSM
8026.5 c	11821.3a	10296.5 b	24707.15*	NGM
22.27b	28.16 a	25.19ab	59.89**	NSM
4400.1 b	4997.5 a	4712a	613696.30**	Y
12527.5b	14262 a	12597.2 b	673965.33**	BY
185.33 a	190.87 a	188 a	53.17^{ns}	DM
89.5 a	97.5 a	93.27 a	8.78^{ns}	DF
135.33 a	140.87 a	138.06 a	53.06^{ns}	DP
50.44 a	43.29 a	44.77 a	11.40^{ns}	PG

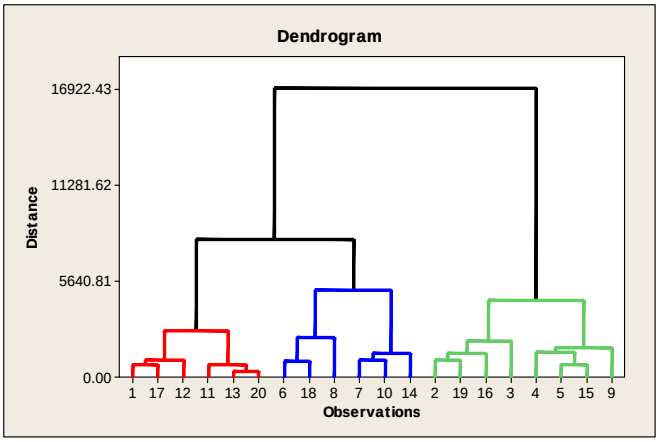

Fig.2Final Dendrogram of 20genotypes of barley ward method

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Table.3correlations between traits

-	PH	PL	LSA	LS	WTG	FLN	NSS	HI	NSSM	NGM	NSM	Y	BY	DM	DF	DP	PG
PH	1	-0.24	-0.22	0.10	-0.06	0.41*	-0.11	0.12	-0.05	0.15	0.01	0.15	-0.09	-0.21	-0.15	-0.28	-0.01
PL		1	0.14	-0.29	0.16	-0.28	0.11	0.31	-0.11	0.06	-0.00	-0.02	-0.29	0.08	0.04	0.08	0.03
LSA			1	0.11	0.30	0.11	0.18	-0.12	0.11	-0.22	0.16	0.08	0.13	-0.00	0.02	-0.01	-0.06
LS				1	0.04	0.63**	0.45*	0.05	0.08	-0.02	0.32	0.28	0.10	-0.36	-0.43	-0.36	-0.38*
WTG					1	-0.00	0.2	-0.04	0.04	-0.49*	-0.09	-0.05	-0.01	-0.66**	-0.51**	-0.66**	0.28
FLN						1	-0.01	0.31	-0.18	0.05	0.12	0.19	-0.16	-0.29	-0.20	-0.28	0.02
NSS							1	-0.11	0.18	0.01	0.22	0.14	0.11	-0.22	-0.20	-0.22	0.10
HI								1	-0.64**	0.16	0.30	0.25	-0.65**	-0.14	0.01	-0.14	-0.22
NSSM									1	0.26	0.41*	0.53*	0.91**	0.20	0.14	0.20	-0.01
NGM										1	0.60**	0.64**	0.38*	0.51**	0.51**	0.51**	-0.41*
NSM											1	0.96**	0.41*	0.08	0.16	0.08	-0.33
Y												1	0.55*	0.05	0.14	0.05	-0.33
BY													1	0.19	0.13	0.19	-0.02
DM														1	0.91**	0.99**	-0.53*
DF															1	0.91**	-0.83**
DP																1	-0.53*
PG																	1

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Antioxidant effect of grape and garlic extracts on the quality of marinated kilka (*Clupeonella cultriventris*) during storage

Shirin Hasani[1], and, Maryam Hasani[2]

Abstract—This study aimed to determine changes in chemical and sensory properties of marinated kilka (*Clupeonella cultriventris*) that contains grape (8%) and garlic (5%) extracts during storage at 4 ºC. According to results, Higher values for Total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA) were found in samples without extract than those with extract. There was significant difference (p> 0.05) on TBA, TVB-N value and sensory analysis between control and samples with extracts. sensory analyses showed that sensory quality of the marinades was getting decrease while TBA value was getting higher at the end of 75 days at 4°C, but the experimental (with extract) group was good quality after 75 days.

Keywords—antioxidant, marinated kilka, grape extract, garlic extract.

I. INTRODUCTION

The marinating process is one of the oldest methods of preservation of sea products[1]. Marinades are solutions composed of acids, salt, oil, spices that used in order to preserve the raw materials. The aim is not only to prevent microorganism growth but marination is also used to tenderise or to change taste, textural and structural properties of raw material [2].

The inhibitory effects of these substances on bacteria and enzymes increase with concentration but it is not advisable to increase the levels of vinegar and salt to suppress potential spoilage problems since they can cause inconsumable taste. Marinated fishes are typically consumed as ready to eat products with no heat treatment [3],[4] and have a limited shelf life [2],[5].

Sea foods contain high concentrations of poly unsaturated fatty acids (PUFA), eicosapentaenoic acid and docosahexaenoic acid. So, sea foods are very susceptible to loss quality during lipid oxidation [6]. Various synthetic and natural antioxidants are used to prevent oxidation of lipids in sea foods for long storage [7]. Grape polyphenolics are well-known antioxidants. Grape skin and seeds are rich source of phenolic compounds. The antioxidant activity of grape has been positively associated with their composition such as anthocyanins, flavonols, flavan-3 ols, procyanidins and phenolic acids [8],[9]. Additionally, these compounds have been shown to reduce hydro peroxide formation and inhibit lipid and protein oxidation [10].in addition to antioxidant effect, grape phenolics have been reported to apply antimicrobial, anti- inflammatory and anti- aging activities [9],[11],[12],[14].

Garlic has an antioxidant activity and antibacterial, antifungal and antiprotozoal effect [15] that used as a flavor enhancement.

The study was, therefore, aimed to determine the changes in chemical and sensory properties of marinated and to determine the antioxidant effect of grape and garlic extracts during the storage period at 4 °C.

II. MATERIALS AND METHODS

Raw material

Kilka *(Clupeonella cultriventris)* was purchased from fisherman in Babolsar seaport in Iran. The fishes were directly transferred to the laboratory in boxes with ice. The fishes were eviscerated, filleted and washed. red grapes prepared freshly, transferred to laboratory and stored in freezer (-18°C). Grape pomaces were dried at 45°C for 72 h, milled to particle size less than 0.5 mm. The fresh garlic was purchased from a local market.

Marinating process

Fishes were immersed into a solution consisting of 30 g/L acetic acid and 150 g/L NaCl. The ratio of fish to solution was 1:1.5 (w: v) [16]. The immersing process was performed at ambient temperature (20 ± 2 c). The maturation process was completed within 30 h, the marinated fish was removed from the solution and put into glass jars. Jars were divided in to three groups. The first group of jars was without any extract, and the second group with 8% grape extract and the third with 5% garlic. All samples were stored at 4 °C and analyzed to determine the quality changes during 75 days.

Extraction

Fresh red grapes (*Vitis vinifera*) were prepared from Shahryar (Iran) and were transferred to laboratory. They were stored at -20 °C until was made into pomace. Grape

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pomaces were dried at 45°C for 72 h, milled to particle size less than 0.5 mm. Dried grape pomace (200 mg) was placed in a test tube, then 20 ml Diethyl ether containing 1% acetic acid was added for removing of pigments and fat. The solution was thoroughly shaken at room temperature for 20 min and centrifuged at 3,000g at 4°C for 10 min, and the supernatant was recovered. Ten ml of Acetone 70% (V/V) were added to the residue, and shaking and centrifugation were repeated. Extractions were performed to calculate the total phenolic content [17].

In the preparation of aqueous extracts, sterile distilled water was used as solvent. For the preparation of aqueous extracts 30 gr of crushed fresh garlic were weighed. 100 cc of sterilled water with a temperature of 70-80 c was added to the flask containing garlic. Flask was placed in a water bath with a temperature of 60 degrees and removed after 24 hours. And the mixture was filtered with a butcher funnel. Approximately 50 ml of extract was obtained [5].

Chemical composition analysis

Proximal composition of fillets crude Protein (N×6.25) and ash content (550 °C) were measured by triplicate [18].Total lipid content [19] and moisture (drying at 105 °C – 24 h) were determined by triplicate [18].

pH

The sample was thoroughly homogenized with 10 mL of distilled water and the homogenate was used for determination of the pH using a digital pH-meter (model Wagtech- cyber scan 510, Germany) [17].

Thiobarbituric acid value (TBA)

The thiobarbituric acid value (as malonaldehyde) was determined calorimetrically by the method of [17]. Absorbance (As) was measured at 530 nm against water blank. A reagent blank was run and absorbance (Ab) recorded. TBA Value (mg of malonaldehyde/kg of tissue) was obtained by the formula.

$$TBA = As\text{-}Ab \times 50/200$$

Total volatile basic nitrogen value (TVB-N)

The total volatile base-nitrogen (TVB-N) content of samples were determined after steam distillation according to the method of AOAC [20]. The analysis was based on titration 0.1 N HCl, using a solution of a boric acid. The results expressed as milligram TVB-N per 100 g muscle.

Sensory analysis

Sensory analysis was performed by a panel of five panelists. The panelists were from the staff of Food Engineering Department who had experience in evaluating seafood. The panelists evaluated the samples for odour, appearance and taste on a nine-point hedonic scale [21]. A score of 9–7 indicated ‘‘very good’’, a score of 6.9–4.0 ‘‘good’’, a score of 3.9–1.0 denoted as spoiled.

Statistical analysis

Three replications of the experiment were conducted at separate times and all analyses were performed in triplicates. Means and standard errors were calculated. Data were analyzed by a split plot design in a completely randomized system, with treatments as a whole plot and storage time and treatments by storage time as a sub-plot. Data analyses were performed using the Procedure of SPSS version 15.0 software.

III. RESULTS AND DISCUSSION

Proximate composition

The results of composition analysis of all samples were shown in Table 1. Moisture content of raw kilka was determined as 74.4±0.26 % but moisture content in the control and experimental groups with grape and garlic extract decreased to 71.63± 0.2, 73.83± 0.33 and 73.77± 0.15, respectively. After marinating process, Protein, Fat and ash content of marinated fish and experimental groups also increased as compared to the content of raw kilka (table 1).

Table 1. Proximate compounds of raw and marinated kilka

Material	Ash (%)	Moisture (%)	Fat (%)	Protein (%)
Raw Kilka	2.80±0.06	74.40±0.26	8.63±0.07	17.63±0.03
Marinade kilka	2.87±0.03	71.63±0.20	9.37±0.07	18.23±0.15
With 8%Grape extract	2.80±0.06	73.83±0.33	9.43±0.03	18.53±0.09
With 5%Garlic extract	2.90±0.06	73.77±0.15	9.33±0.09	18.57±0.18

Values are means ± standard of mean of three replicate Determinations

Physical and chemical quality analysis

pH is an important and effective indicator of meat quality [22]. Batista and Morao de Campos (1992) found a pH value of 6.1 in sardine [23]. The pH value in sardine found by El Marrackchi [24], Varlyk [25] and was 5.83 and 6.35 respectively. But the pH values do not show a certain time of spoilage and other chemical and sensory analyses supported it [26] pH value of raw material used in this study was 6.83 after marinating, the pH value decreased to 4.2 at the beginning of the storage and at the end of the storage period the pH in experimental groups with grape and garlic extract decreased to 3.9 and 4.13, respectively. As soon as the marinating bath comes in to contact with the fish, a

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diffusion of acetic acid and salt perform in to the tissue of the fish flesh until reached to a concentration balance [27]. Table 2 shows the change in pH value. Lower pH in the samples treated with extracts can be attributed to the anti bacterial extracts property [28].

Table 2. Changes in pH (ppm) values of marinades in different times of storaging

Time (Day)	Control	Grape extract in marinade	Garlic extract in marinade
0	4.2±0.06 A a	4.2±0.10 A ab	4.2±0.03 A abc
15	4.4±0.10 A a	4.2±0 A ab	4.36±0.03 A a
30	4.36±0.12 A a	4.2±0.06 A ab	4.2±0.06 A bc
45	4.43±0.03 A a	4.3±0.06 A a	4.36±0.09 A a
60	4.3±0 A a	4.06±0.03 B bc	4.3±0 A ab
75	4.2±0.06 A a	3.9±0.06 B c	4.13±0.03 A c

The values are expressed as mean ± standard deviation, n=3. Values in a same column followed by different letters (a–f) indicate significant differences ($p<0.05$) during storage periods. Values in a same row followed by different letters (A–C) indicate significant differences of the parameter with respect to the extract treatment ($p<0.05$).

The highly unsaturated lipids in fat-rich fish are susceptible to oxidation and results a rancidity and changes smell, taste, texture, color and nutritional value [26]. TBA is a good indicator of the quality and degree of lipid oxidation of the fish whether it was frozen, chilled or stored in ice [29]. It has been suggested that a maximum TBA value, indicating the good quality of the fish is 5 mg malonaldehyde/kg while fish may be consumed up to a TBA value of 8 mg malonaldehyde (MA)/kg [25]. At the beginning of the storage period TBA values of the control and experimental groups with grape and garlic extract were 0.92±0.02, 0.53 ±0.04 and 0.57±0.07 mg malonaldehyde/kg, respectively. Whereas at the end of the storage they reached 6.71±0.06, 2.26 ±0.09 and 2.36±0.09 mg malonaldehyde/kg, respectively. In these studies, it was shown the use of extract at different concentration prevented lipid oxidation in fish minces. As shown in table 3 TBA values of experimental groups stays at high quality to the end of storage time and in the control until the 60th day were high quality. Whereas, the value reached to consumption limitation the end of the storage at day 75.

Table 3. Changes in TBA (mg malondialdehyde/ g meat) values of marinades in different times of storaging

Time (Day)	Control	Grape extract in marinade	Garlic extract in marinade
0	0.92±0.02 A f	0.53±0.04 B e	0.57±0.07 B d
15	1.41±0.06 A e	0.61±0.06 B de	0.7±0 B d
30	2.13±0.12 A d	0.76±0.03 B d	0.85±0.03 B cd
45	3.16±0.18 A c	1.01±0.04 B c	1.06±0.12 B c
60	3.96±0.15 A b	1.53±0.09 B b	1.53±0.15 B b
75	6.71±0.06 A a	2.26±0.09 B a	2.36±0.09 B a

The values are expressed as mean ± standard deviation, n=3. Values in a same column followed by different letters (a–f) indicate significant differences ($p<0.05$) during storage periods. Values in a same row followed by different letters (A–C) indicate significant differences of the parameter with respect to the extract treatment ($p<0.05$).

Table 4. Changes in TVB-N (mg N/100 g fish flesh) values of marinades in different times of storaging

Time (Day)	Control	Grape extract in marinade	Garlic extract in marinade
0	7.73±0.12 A d	7.33±0.17 AB c	7.07±0.07 B c
15	8.1±0.21 A cd	7.4±0.10 B bc	6.83±0.17 B bc
30	8.7±0.36 A c	7.37±0.12 B bc	7.1±0.06 B bc
45	10.8±0.44 A b	7.77±0.19 B bc	7.33±0.07 B b
60	11.47±0.35 A ab	7.9±0.15 B ab	7.77±0.15 B a
75	12.23±0.12 A a	8.4±0.23 B a	7.97±0.03 B a

The values are expressed as mean ± standard deviation, n=3. Values in a same column followed by different letters (a–f) indicate significant differences ($p<0.05$) during storage periods. Values in a same row followed by different letters (A–C) indicate significant differences of the parameter with respect to the extract treatment ($p<0.05$).

TVB-N is used to determinate of the spoilage level during storage period [30]. It is reported that TVB-N value is affected by species, change season and region, age and sex of fish. The limit of acceptability of TVB-N in fish and fish products is 30-35 mg/100 gr (European Economic Comission, 1995). TVB-N value increased significantly In both control and experimental groups during storage. The differences between control and experimental group marinades were found to be significant ($p<0.05$). At the end of the storage period TVB-N value in control and experimental groups with grape and garlic extract increased 12.23±0.12, 8.4±0.23 and 7.97±0.03, respectively. In one report TVB-N value of 8.31 mg/100 gr in anchovy marinated using 2% acetic acid increased to 15.18 mg/100 gr [31]. In this study, both control and experimental groups still acceptable for consumption at the end of storage period. But the samples with garlic extract had the least value. This study showed that addition of grape or garlic extract decreased TVB-N value significantly during storage at 4c° ($p<0.05$).

Sensory analysis

Sensory testing plays an important role in food quality evaluation since the ultimate test of food quality is consumer response [32]. In this study sensory scores of marinated kilka significantly decreased ($p<0.05$) throughout the storage. Sensory evaluations of marinated kilka during the storage are shown in table5. Significant differences were determined in the control and experimental groups during storage ($p<0.05$). The control was the third quality and the panelists recognized rancidity at day 75. The experimental group still were very good during the entire period and no

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significant differences ($p>0.05$) between the experimental groups were determined during the storage.

Table 5. Sensory analysis

Time (Day)	Control	Grape extract in marinade	Garlic extract in marinade
0	9.±0 A a	9±0 A a	8.93±0.03 A a
15	8.70±0.06 B b	9±0 A a	8.93±0.03 A a
30	8.17±0.03 C c	9±0 A a	8.77±0.03 B b
45	7.90±0.06 B d	8.73±0.03 A b	8.70±0.06 A b
60	7.07±0.03 B e	8.53±0.03 A c	8.47±0.03 A c
75	6.03±0.03 B f	8±0 A d	8±0 A d

Values are means ± standard of mean of three replicate Determinations

IV. CONCLUSION

In conclusion according to the sensory, TBA and TVB-N analysis, grape and garlic extract at the level used rendered antioxidant effect and helped to prolong the shelf life of marinated fish.

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Study of the Effect of Plant Growth Regulators and Explants Type on In vitro Regeneration of Stevia (Stevia rebaudiana)

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Abstract
Stevia (*Stevia rebaudiana*) is a perennial, herbaceous plants belonging to the family of Asteraceae, The it have great economic and medical importance, because this plant have high sweeteners and non-caloric property. Genetic engineering of stevia requires to efficient method and effective tissue culture. Regeneration from tissue culture is one of the suitable methods for large scale production and genetic engineering plants. In this investigation were studied effective factors on regeneration of stevia such as explants type and different concentrations of growth regulators in order to achieve efficient protocol. For this purpose, different concentrations of TDZ (0, 0.5, 1, 1.5 and 2 mg/l) and NAA (0, 0.1, 0.5 and 1 mg/l) with two explants (Leaf and Internode) were used. After 4 weeks number of regeneration were evaluated. The results showed that the regeneration was increased with increasing TDZ concentration. The highest percentage and number of regeneration obtained in leaf explants with 1.5 mg/l TDZ and 0 mg/l NAA.

Key words: Explant, Plant Growth Regulator, Regeneration, Stevia

Introduction

Medicinal plants are of great interest to researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds [13]. Stevia (*Stevia rebaudiana* Bertoni) is a perennial sweet herb, belonging to the family Asteraceae. This plant is indigenous to the Rio Monday valley of the Amambay mountain region in Paraguay. Its leaves contain approximately 10% of steviosides which are intensely sweet compounds (150 to 300 times sweeter than sugar) [7].

The leaves have been traditionally used for hundreds of years in Paraguay and Brazil to sweeten local teas, medicines and as a 'sweet treat'. Japan is now the largest consumer of steviosides extracted from stevia leaves; in japan stevia replaces the chemical sweeteners, aspartame etc, which were banned there in the 1970's. Other countries use lesser quantities of steviosides. Steviosides have zero calories and can be used wherever sugar is used, including in baking etc [5].

It is a sweet herb, which contains natural non-caloric sweetener. It is of immense value due to its adaptability to wide climatic range, the high sweet content, and its significant contribution to the welfare of human life. This over's a solution for complex diabetic problems and obesity in humans, being calorie free [2]. The worldwide demand for high potency sweeteners, particularly natural sweeteners, is expected to increase in the years to come. It is being commercially cultivated in China, Taiwan, Thailand, Korea, Japan, India and Malaysia. In addition, stevia possesses hypoglycemic, hypotensive, vasodilating, taste improving, sweetening, antimicrobial properties and increases urination function of the body. It has been found to be non-toxic, non-carcinogenic, non-mutagenic and is devoid of genotoxic effect [6].

Stevia is a valuable medicinal plant species and it is being used for the treatment of diabetes. It does not affect blood sugar level hence safe for diabetics. The key benefit of Stevia is it stimulates the release of insulin and normalizes blood glucose levels. It is recommended for diabetes and has been extensively tested on animals and has been used by humans with no side effects. Stevia extract and stevioside are officially approved as food additives in Brazil, Korea and Japan. Stevia could be used as a major source of high potency sweetener (alternate to sucrose) in the near future [3].

Currently, there is a high demand for raw material of this medicinal herb due to ever increasing diabetes disorder

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among the population. In order to meet the increased demand an efficient in vitro propagation of S. rebaudiana was established. Although stevia can be helpful to anyone, there are certain groups who are more likely to benefit from its remarkable sweetening potential [1]. These include diabetic patients, those interested in decreasing caloric intake, and children. Stevia is a small perennial shrub that has been used for centuries as a bio-sweetener and for other medicinal uses such as to lower blood sugar. Its white crystalline compound (stevioside) is the natural herbal sweetener with no calories and is over 100-300 times sweeter than table sugar [4].

Stevia is a herb that is used extensively in various areas of the world as a non-caloric sugar substitute. Due to its huge applications in food, drugs and pharmaceutical industries, it is now commercially cultivated in many countries of the world viz., Brazil, Paraguay, Uruguay, Central America, Thailand, China and Japan [11].

Stevia can be propagated by seed and by stem cuttings. at the first step low viability and low seed germination, limit their extensive cultivation. Currently, stevia is being propagated by stem cuttings. Low seed germination percentage is a major limiting factor for large scale cultivation of Stevia plant species for commercial usage [12]. Further vegetative propagation is also limited by the less number of individuals obtained from single plant. Therefore, a suitable alternative method for large scale plant production within a short period is the use of in vitro culture technology [10].

One of the effective methods to produce this plant is in vitro technology that solves the problem of large scale production and effective researches. Because of this fact the providing a suitable tissue culture protocol is the first steps in large scale production as well as genetic engineering. Regeneration from tissue culture is one of the suitable methods for large scale production and genetic engineering plants [13].

Genetic engineering of stevia requires to efficient method and effective tissue culture. Regeneration from tissue culture is one of the suitable methods for large scale production and genetic engineering plants [9]. The optimization of tissue culture to reach the objective parameters in stevia was investigated. The present study aimed to investigate the effects of different concentrations of growth regulators such as auxin and cytokinins to identify the best hormonal concentration to obtain the highest regeneration in vitro produced plantlets using leaf and internode explants.

Materials and methods

In this investigation were studied effective factors on regeneration of stevia such as explant type and different concentrations of growth regulators from achieve efficient protocol. in order to different concentrations of TDZ and NAA with two explants were used. The culture medium consisted of MS [8] salts, vitamins, 3% (w/v) sucrose and the pH of the media was adjusted to 5.6 with 0.1 N NaOH or HCl before adding of 0.8% (w/v) agar and autoclaved at 121 ◦C for 15 min. After surface sterilized Leaf and Internode explants were cultured on MS medium supplemented with different concentrations of TDZ (0, 0.5, 1, 1.5 and 2 mg/l) and NAA (0, 0.1, 0.5 and 1 mg/l) for regeneration. The cultures were incubated at 24±2 ◦C under 16/8 h (light/dark cycle) photoperiod (60 µE m−2 s−1) and irradiance provided by cool-white fluorescent tubes. After 4 weeks number of regeneration were evaluated. Also, the elongated shoots were transferred onto half-strength MS medium fortified with 2 mg/l NAA for root induction. Plantlets with well-developed roots were removed from the culture tubes and gently washed under running tap water to remove adhering medium. Subsequently, they were transferred to plastic cups containing sterile sand and soil mixture. The potted plantlets were initially maintained in the controlled environment for two weeks and subsequently they were shifted to the greenhouse. After thirty days, the plantlets were successfully established in the field. Experiment was conducted in factorial based on completely randomized design (CRD) with 3 replications and observations was carried out after the 4 weeks. The analysis of variance (ANOVA) was performed using SAS programme. The differences among means were determined by Dunkan Test at 1% significant level.

Results and Discussion

In vitro propagation has facilitated rapid and mass multiplication of diseases-free plants and acts as a new tool for modern breeding through genetic manipulation. The impact of different concentrations of TDZ (0, 0.5, 1, 1.5 and 2 mg/l) and NAA (0, 0.1, 0.5 and 1 mg/l) were examined on regeneration capacity in vitro condition from Leaf and Internode explants. The statistical analysis of variation for shoot regeneration rate per explant showed significant difference for hormone and explants type.

Interactive source variation of TDZ × NAA × explants showed significance at $p<0.01$ for shoot regeneration rate per explant in vitro conditin (Table 1). Results of shoot regeneration rate were illustrated on figure 1. As indicated on this figure1, The type of explant markedly influenced organogenesis and growth of the regenerated shoots. So that the regeneration frequencies were higher with leaf explants. Also, the regeneration frequencies was increased with increasing TDZ concentration and decreased with increasing NAA concentration . The highest percentage and number of shoot regeneration obtained in leaf explant with 1.5 mg/l TDZ and 0.0 mg/l NAA.

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Table 1: Analysis of variance (ANOVA) for shoot regeneration from explants type of *Stevia rebaudiana*

Source of variance	Degrees of freedom	Mean square regeneratio
TDZ	3	27/18**
NAA	3	34/40**
Explant	1	48/16**
TDZ x NAA	9	7/17**
TDZ x Explant	3	7/02**
NAA x Explant	3	12/08**
TDZ x NAA x Explant	9	1/63**
Error	62	0/13
٪CV		8/3

**: Significant at 1٪ probability level

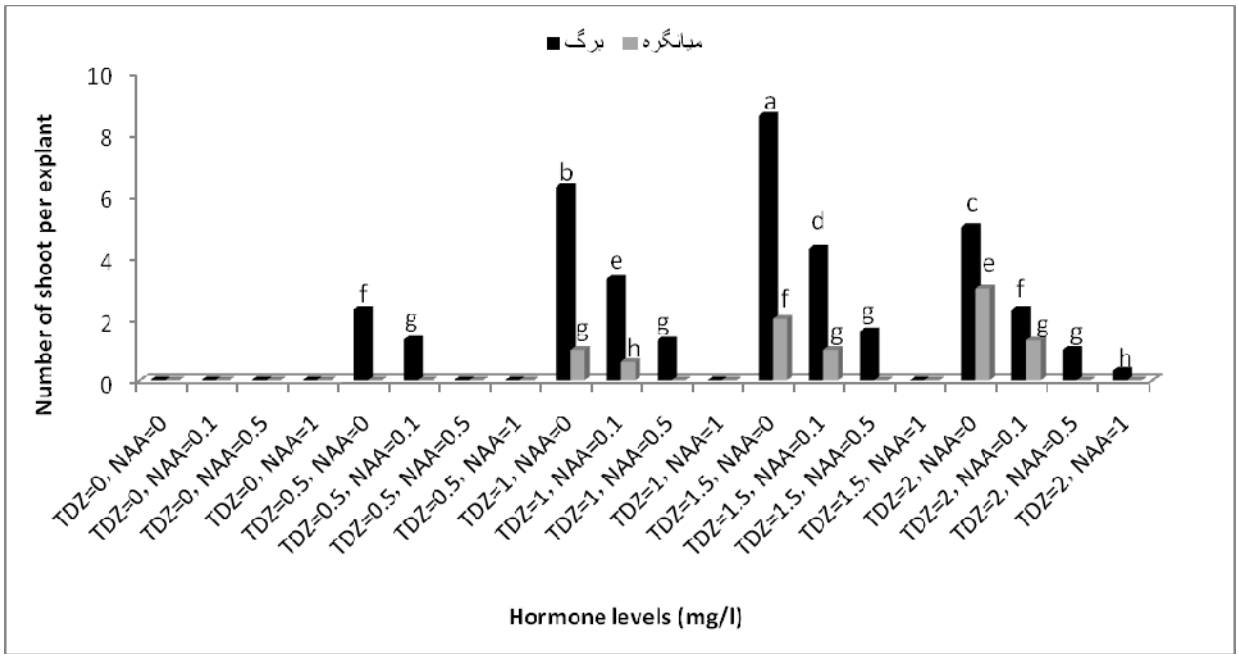

Fig. 1. Effect of different concentrations TDZ and NAA on shoot regeneration from explants type of *Stevia rebaudiana*

Conclusion

The present study was conducted to optimize an efficient and reproducible regeneration protocol from leaf explants with the aim of using it for Agrobacterium-mediated gene transfer experiments in stevia. Genetic transformation to the

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plants requires plant regeneration technique that results in production of large number of shoots and allows the faithful multiplication of transferred gene without causing variations. Adventitious shoot formation is a reliable technique for clonal propagation as it prevents somaclonal variations in the cultures. The type of tissue or explant used for clonal multiplication also influences the chances of genetic variation. Because of the non-uniform nature of callus tissue, genetic mutations are more frequent in shoots regenerated from callus, particularly with prolonged subculturing, than from other types of tissues. Adventitious shoot regeneration is most preferred if Agrobacterium-mediated gene transfer is to be achieved and leaf explants are best suited for both adventitious shoot formation and Agrobacterium-mediated gene transfer experiments.

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Effects of Agropyron elongatum drilling operations with contour furrows on the production and vegetation in semi-arid rangelands (case study Ghochan city, region Bharkysh).

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Abstract— This study aims to evaluate the corrective actions contour farrows with drilling impact on productivity and vegetation in Bharkysh Ghochan range. For systematic random sampling method, used the six transects 100m at each site based corrective and control area and along each transect Was used ten plot $2m^2$ for harvest vegetation information. Comparisons showed that rangeland statistically significant difference in the level of a percent**.**

Keywords—contour farrows, drilling, Corrective Actions, Bharkysh Ghochan range

INTRODUCTION

Range As one of the land use and a series of physical and biological factors that provide Forage needed for livestock .also Support To produce the known proteins that involved in the regulation of water regime , moderating the weather, the needed for medical and industrial , recreational , Beauty , and as a genetic resource will complete its role in nature [1]. Ranges play an important role in protecting soil and water supply needed protein, medicinal plants and industrial production hence requires serious attention. Ecological and economic value of natural resources primarily dependent on forage productivity and efficiency in water and soil conservation, so that In a measure of the range of two important factors that represent ecological and economic value of pasture areas, the coverage and production[8]. In many parts destroyed valuable species and the soil has been exposed to water and wind erosion . For Rangeland revive and Correction have the different methods, such as planting , seeding with operations such as furrows , pitting , etc, can be used to return to his previous position of the regions [3]. If pasture soil for various reasons, lose their infiltration And naturally not be able to absorb and hold water from rainfall, In the short term by running a series of special mechanical methods to suit local conditions, allowing water to infiltrate in the soil . During this procedure the additional water into the soil, This water is used to enhance vegetation. After that time, the mechanical operation efficiency decreases, Vegetation created a duty to prevent surface runoff and infiltrationin to the soil consistently performs [2]. Rangeland improvment and revive activities to increase forage production, species diversity and ecosystem stability is achieved, Therefore Study of the positive and negative effects on species diversity of grassland reform activities is necessary. Often improvement activities is done in the mountain ranges of the species planted in small pits(hilling) or create a contoured grooves (contour farrows) [14]. contour farrows creation is the creation of shallow furrows on the surface of contoured pastures. The goal of contour farrowsas a kind of corrective action in pastures , storage of rainfall in the soil and use excess moisture Collected in the Farrow for the growth of desirable species .In addition Surface runoff is controlled,. So the important point that should be considered in site selection for establishing contour farrows, In addition, slope ,is runoff, soil fertility and natural talent. In this method , in the 1930s in large scale and high diversity in terms of size and distance between farrows has been implemented [3].

Theory and literature

[11] studied the effect contour farrows, pitting and ripping on rangelands in the West, the United States showed that the Farrow and pitting operations and culture of Agropyron desertorum after the contour farrows Due to the increase in soil moisture and infiltration Forage production was increased significantly, especially in favorable soil[12] Found that ripping operations on six regions in West America Production of perennial grasses has reduced, Due to low-level manipulation of soil, so that water infiltration is not increased.[17] Concluded that contour farrows in pastures in South East Mantana, infiltration of soil and forage production has increased. [13] concluded that Farrow in one area of Wyoming on forage production for 35 years has had a positive

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effect. [18] stated that the West Utah grazing Capacity in the pasture was seeding From 3/1 to 2/8 animal units per acres, with an average of 8/3 has changed. In a period of 8 years [20], with studies on rangeland habitats pan (layer) of hard, reported that contour farrows has increased average the forage production up to165%And soil water availability to 107 percent, but the total coverage The range is reduced to 73%. The study pastures Wyoming USA by [11], observed that the natural grasslands, create the Farrow has been able to reduce the Surface flow of water About 84 to 94 percent and protection of the Lower land from the Accumulation of sediment and surface water.

Materials and Methods

Bharkysh is located in south side of the city Ghochan, central part of the village Dughaie. Bharkysh Approximate distance to the Ghochan city center, about 73 kilometers and at longitude 58°36́ 42́, 58° 40́ 00́ and latitude36°42́ 10́, 36° 44́ 29́ is located. The project area in terms of climate. Located in a Consists of arid to semi arid cold. The average annual rainfall is about 346mm, the minimum and maximum annual absolute temperature, respectively, and -23/5 to +39/5C and humidity average in about 53/4 per cent. For sampling using the systematic random method, Six transects 100 meters were established at each site corrective and control area, And throughout each transect using a ten plot2m^2. Vegetation data were collected With in each plot listing of existing canopy cover, density and total production was determined. After ensuring the normal distribution of data to compare the characteristics of vegetation corrective action sites with control sites, independent t-test was used[4].

Results and discussion

In order to identify plant species in the study sites, List of species identified and prepared Which is considered very important in the study of vegetation. Floristic list of classes and palatability of each species in the study sites(Table 1)are presented.

Table1-List of Plants and class palatability of the study sites

Control sit	Farrow sit
Acantholimon raddeanum (III)	*Acantholimon raddeanum* (III)
Acantholimon raddeanum (III)	*Acantholimon sp* (III)
Acantholimon sp (III)	*Agelipos sp* (II)
Acanthophyllum crasinodum (III)	*Agropyron elongatum* (II)
Acanthophyllumglandulosum (III)	*Medicagorigidula*(I)
Achillea wilhelmsii (II)	*Medicago sativa* (I)
Acroptilon repens (III)	*Onobrychis sativa* (I)
Aegilops tauschii(II)	*Poa bulbosa*(II)
Arehenatherum kotschyi (II)	*Trifolium spp*(I)
Artemisia auchri (III)	*Bromus dantoniae*II
Artemisia scoparica (II)	*Bromus tectorum*(III)
Astragalus brevidens (I)	

In Table 2, descriptive information on the characteristics of vegetation(Canopy, production) study sites with the control site.Results Table 2 shows the study site with control site, the vegetation cover Characteristics includes of production and Canopy percent are significantly different at the one percent level, So that the Canopy cover percentage, production in the study site compared with the control site increased.

Table 2-Descriptive information on vegetation characteristics of the study site with control site.

Total production(kg/ha)		**Canopy%**		**Topic**
Std error	average	Std error	average	**site**
2/45	461/01	1/03	68/23	**Contour farrow**
·/96	153/66	2/17	46/03	**control**

Results were analyzed using t-test which the results in Figures 1, 2 are presented.T-test results indicate that the properties of the vegetation in the Contour farrow site has a significant

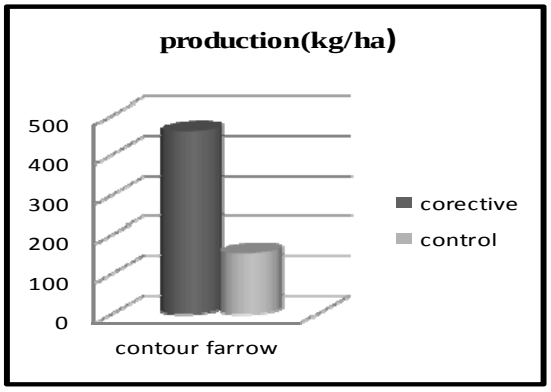

Figure 1- Comparison of the average production Farrow site with the control site

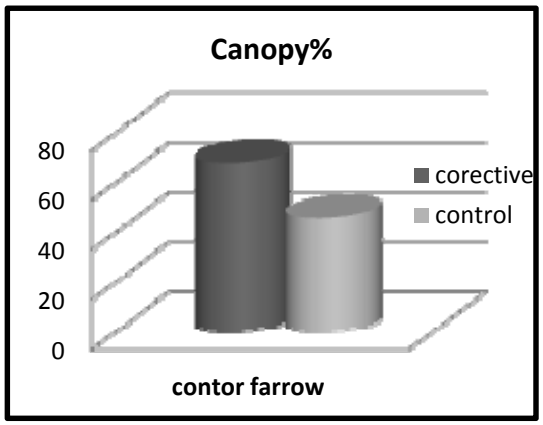

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Figure 2- Comparison of mean percent Canopy Farrow site and control site.

Conclusions andrecommendations

In contour farrows sit Canopy cover percentage(1/48 equal) and production(3/21 equal) than control site have increased significantly. In other words,Because of contour farrows Vegetation zone to the climax has gone and the percentage of non-palatable species composition contour farrows area is reduced. In control site, soil and vegetation degradation and absence of appropriate conditions, Annuals and shrub species that mostly Invasive species are non-palatable Inthe region have developed .The results with the findings of [19], [15], [16], [6], [5], adapted to be. According to the Agropyron elongatum -resistant species to drought and salinity and the power of survival is high [9] , so widely spread in the region and the majority of class I and percentage of vegetation cover on site contour farrows related to species Agropyron elongatum . in rangelands that the filing of rainfall Like contour farrows pitting,banket ,correction and regeneration of the grasslands, such as Planted and seeding takes place. a result Water storage and increase infiltration and creating moisture sufficient Due to Water storage and improve the physical and chemical properties of soil, Located in an area of significant vegetation and improve grassland Can have a positive impact properties of vegetation and pasture condition improvement[7]. Similar results[13] in Wyoming get built contour farrows. [11] showed that the effect of contour farrows, pitting and culture of Agropyron desertorum after contour farrows,the effect of soil moisture and Its deep penetration,Forage production had increased significantly, especially in Favorable soil. [10] have shown that the mechanical operation contour farrows in pastures of South East Mantana,Infiltration of the soil and forage production has increased[17]. Sandy loam soils in America Wyoming Reached to a similar conclusion and reported increase infiltration rates were as a reason to increase forage production. Suggested to be due to the positive effect the contour farrows on grassland area Be done in similar areas of the region Thus, we have further forage production and improve rangeland.

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Study Effect Entrepreneurship Development on Sustainable Development Rural

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Abstract

One of the most effective factors in rural development is entrepreneurship, because this can have a significant role on promotion and life quality improvement in rural's lives with creating new job opportunities. Entrepreneurship is an activity that can somehow solve the major problems in rural areas like unemployment, low income, lack of diversity in economics, etc. And in final to prevent the villagers not to immigrate to cities or metropolitan cities, besides this can have a positive effect on the other aspects of their human living. Therefore, we conclude that rural entrepreneurship is a new solution in theories of empowerment development and capacity building in rural areas in order to reduce the rural and urban gap, having an equal economy, social and an institutional environment as an important tool in stable rural development. The main purpose of this article is to research on the effect of entrepreneurship development on stable rural development in rural areas. Information in this survey is a library kind and it is documentary. Results of this survey demonstrate that the most important solution in Sustainable rural development is to create entrepreneurship based on capacities and potentials in every rural to achieve a better economy. In conclusion increasing on all the aspects of life quality in rural areas with use of capacities and potentials corresponded with the same rural, and in the end it leads to the substantiation of stable rural development.

Key words: entrepreneurship development, rural, Sustainable rural development.

1. Introduction

Entrepreneurship is a concept that has existed since the beginning of human creation. Entrepreneurship is considered as the centre and gravity of effort and development in modernity age. Regardless of "Entrepreneurship development" category, we cannot achieve the growth index and development; this can be the outcome of efficiency technical and industrial change in economical field.

In transition of traditional stage to industrial stage, we must draw a particular attention on entrepreneur's individual abilities and capabilities in utilization of natural sources and modern technologies owning to using the new strategies in business, so that they could have an optimum use of tools and achieve to the desirable quality of goods with available services. [1] In total, identifying exclusive features of entrepreneur and effective factors on entrepreneurship cultural formation in society, and also in person's job in contemporary age can be considered as elements of growth and development through different dimensions of economical, cultural, social and political. So that entrepreneurship element is considered as the motor of production and economical development and an amplifier to spirit of investment in manpower [4]. One of the barriers along the way is to have a total realization of stable rural development goals on minor level, and in individual and familial categories. Rural's lack of recognition and knowledge toward sources, their possible and capacities, and their potential environment is to please their diverse needs with optimum appropriation and desirable efficiency [8]. Nowadays, in majority of countries there is a particular attention on successful persons and entrepreneurs, innovation reinforcement, successes and a platform in this affair can be counted as tools of economical development especially in developing countries; high effectiveness entrepreneurship activities can result in economical development, occupation, innovation in activities, competitiveness and so on [18]. Therefore this entrepreneurship activities development in rural areas must be based on the existence capacities and potentials in every rural and belong to that village, so that they can accomplish and their affair lead to stable rural development. The basic step is to identify each village's capacities and potentials, and choose the best place for related entrepreneurship settlement activities until they prevent source and time wasting. In this part first we need to bring the entrepreneurship culture into rurals, so we could expect them entrepreneurship. Nowadays we have a special attention in economical theories and spontaneous economical activities in frame of entrepreneurship's rural areas. In this context we have pointed that economical development and entrepreneurship have a close relation, so we conclude that rural entrepreneurship is a new solution in developmental theories to empowerment and capacity building toward achieving stable development. We should know that despite having creative, innovative

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and hardworking persons, living entrepreneurship style is not yet institutionalized in villages. Because there are different barriers and limitations, like unfamiliarity with lack of acceptance and encouragement and also financial agricultural support, suggestions and patterns without previous plans, deficiency of quick reply to ideas and new suggestions and its system, lack of trust on ideas stealing, lack of common innovation prospect, being tension and discontent, senior management's isolation, deficiency of access to authentic information and clear mechanical structure, cultural support absence of entrepreneurs, distance in business and service, gaps in investment access, network and communicative opportunities reduction, lack of industrial catalyst clusters and inner dissociation in rural society, unfamiliarity with knowledge usage, and networks and sources all in all have caused a minor number of entrepreneurs among villagers [11]. As a result, those effective and important political strategies in rural areas can lead to entrepreneurship development. According to entrepreneurship and its development, despite that fact that certain motivation has a positive step in creating a suitable atmosphere to develop, national source increase, reducing unemployment rate will be considered as structural and developmental balance in villages. Therefore, aim of this research is to survey the effect of entrepreneurship development on stable rural development in rurales.

2. Discussion

2-1 The concept of development

In general, development is defined as an effort to life improvement and history of human's society, but in modern definition it is more than just a comparative definition. Development and underdevelopment phenomena have been one of the most debatable topics in range of political economic since the end of World War II [5]. On one hand, we can say that development is to create fundamental changes in social structure and its orientations to a total substantiation of purposes. In this critical transformation if people don't be involve in, the growth fruit will not be their portion [2]. On the other hand, development is not initiated with material goods, but it's started with human's upbringing in discipline structure. Without these three all the sources would remain hidden and useless [3].

2-2 Sustainable development

Sustainable development, not only includes comprehensive flow in population, society, sources and environment, but also it has a more fair complex connection among generations. Therefore, it is necessary to bring all the components in a common and coordinated base.

2-3 Rural development in Iran

Wrong applied politics about rural development planning caused economical poverty, income reducing for work and effort, hidden and obvious unemployment, premature immigrations to cities especially to metropolitan cities, inequality and gap increase between urban and rural areas. Hence, in recent years we have had more focus on social economical development, skills and modern technology ICT; rural development can be pointed at the same time.

2-4 Stable rural development

According to multidimensional nature of stable rural development activities, we can consider them as "comprehensive development" [7]. In recent decades, Iran's rural society has been significantly changed; in near future these changes will continue with further acceleration, but in order to compromise with situations and orientations of rural society, a part of changes has broadly been spontaneous and based on necessities [9]. On the term of development, rural population live in an unstable environment and based on geography's term they live in small scattered residences, with theses two features rural space organizing has faced with challenges. So, we need to have a working procedure to plan a rural society in Iran with these two features [6].

2-5 Entrepreneurship

Entrepreneurship's word comes from a French word *Entreprendre* and it means "to commit", based on this Webster dictionary's definition, entrepreneur is a person who commits to organize and manage the damagers of economical activities. Entrepreneurship is a process of unique formed collection of sources, in order to benefit the chances. We can call the entrepreneurship a process creating value with exclusive sources to benefit a chance.

2-6 Entrepreneurship's culture

The relationship between entrepreneurship and culture is debatable from two aspects. On one hand, results in entrepreneurship effects on society, on the other hand entrepreneurship and its dominated culture is affected by culture's basic society, moreover it can make a fundamental changes too. With more job opportunities in entrepreneurship, wealth and improving the economic situation is an upgrade to background of society's cultural. With meeting the basic subsistence needs there will be a space to great needs and if this be guided to a right way, it will cause human sublimation. Moreover, innovation is one of the fundamental features of entrepreneurship; it will cause to produce more products, and services will be newer and different, thus people's power of choice will increase. With increasing in welfare rates, there will be more peace, free time and job opportunities. All around the world and especially developing countries the main business is in villager's hands and they play the main role in their country's

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economy [15]. However they still have their specifications, like introspection, inflexibility and stability in changes [13]. For this reason, they don't tend to take risks in the entrepreneurship's frame [14]. Most of them have the same old traditional life that it prevents them to have creative innovation and entrepreneurship [12]. Thus, rural entrepreneurship development is a potential to help the diversification in income and production rates in agricultural and none-agricultural products, and it will increase stable food security and provides chances to reduce the subsistence risks in rural areas. Rural entrepreneurship provides more job opportunities in agricultural and none-agricultural parts. In rural entrepreneurship private sector plays an important role as the most important factor of sable economical development in rural areas. Rural entrepreneurship has some basic elements that focus on providing fundamental's rural substructure (software and hardware), and it also focuses on decreasing poverty and increasing their bases of growth [fig 2]

From another point of view, we conclude that for collecting information about organizations, designing entrepreneurship's activities and supporting activates in rural areas, we need four main factors to the spirit of entrepreneurship: 1) creating suitable activities with identified ones of local society 2) enough production with proportional scale, sources and local skills 3) focus on entrepreneurship 4) continues learning via changing previous courses. Hence, we can say that with creating entrepreneurship and employment in rurals, there will be poverty reduction and life quality improvement, and this can prevent villagers to immigrate, so that this decreases rural and urban gap, and finally it will lead to stable rural development.

3. Conclusions and suggestions

Entrepreneurs are the heart of economical, agricultural and especially rural development. Rural development is also a factor of life improvement in low-income stratum, and we should consider the villagers as sufficiency in the total development of the country. So, according to the entrepreneurship's concept and rural development we can figure out the relationship between them. Rural development has a special place in stable development discussion, and an entrepreneur is a person who mobilizes power of sources to respond to a request. He can create anything from nothing. Today's stable development subject is to empower the villagers and this entrepreneurship has gotten the hopes up for its substantiation. Entrepreneurship hopes to solve today's rural problems; like economical, social and subsistence and they have presented some plans to employ them through entrepreneurship. Entrepreneurship is not just to employ majority of rural residents, but it can be a step to rural development, as some pundits say "development itself equals with economical development". Therefore, focusing on establishing rural companies, developing altering industries, developing cooperation and agricultural services would be an appropriated action toward rural development and entrepreneurship. Based on experiences of populated and poor countries that majority of their population is villagers, focusing on entrepreneurship and rural development help the villagers and farmers not to immigrate to cities, and this can bring welfare, job opportunities and development into their own villages. Surveys and studies show that, if welfare facilities and services be provided in rural areas, it will reduce the rate of immigration. We can say that one of the most critical roots of unemployment, irregular growth of population, increasing poverty and corruption in society all are caused by villager's immigration. On the other hand, when young jobless villagers immigrate to find a job, they will face city's challenges, and they will feel disillusioned, plus to meet their needs they might start a mendacious job. Thus, in current situation supporting agricultural, farmers and rural producers with different ways (such financial support, giving long-term low-interest loan), encouraging the top producers and introducing them to other ruralrs, increasing cooperative numbers and encouraging ruralrs to participate, benefit the facilities, supporting entrepreneurs (those who work in different areas like, livestock, supplies, agricultural that create numerous job opportunities for young villagers) are the steps to growth of economical and social field, and sufficiency of production will improve and reduce the unemployment. Rural company's development and supporting them and persons who have new useful designs will create a lot of job opportunities for both young villagers and townies. This solution would have a significant effect not only on entrepreneurship development, but also stable country's development. One of the most effective factors in rural entrepreneurship is to empower villagers, and motivate them to self-employment and entrepreneurship. If rurales that cover the majority of rural population and countries at the same time be motivated to entrepreneurship, entrepreneurship's spirit and social cooperative will boost in them. Stable rural development that is a sub branch of stable country development would verify much faster. As cities entrepreneurship development in rural areas needs to emphasize on three basic prerequisites:

A. Entrepreneurship's culture development

Entrepreneurship's culture is kind of social culture that encourages and supports entrepreneurship's behavior.

B. Training entrepreneurship

Starting and managing an active economical unite in villages need familiarity with a wide range of knowledge and skills. Young villagers usually have the least skills in this filed. Holding entrepreneurship

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training courses and consulting is the general policy of entrepreneurship development in villages.

C. Entrepreneurship's infrastructure development

Providing an access to fund especially risky funds, giving loan to entrepreneurs, developing transportation's facilities, extending informative and communicative systems, connecting to information sources and general knowledge, extending life facilities in villages, and so on will create the entrepreneurship development in rurales.

Long-term strategies in rural entrepreneurship development include:

1) Creating a particular situation to make the immigration process reverse
2) Correcting and changing the distribution pattern and energy's consumption
3) Developing and optimizing transportation's network
4) Prohibiting changing user's garden lands, farms and pasture fields
5) Improving management and correcting strategies in agricultural production
6) Developing early return altering industries in disposed areas
7) Economizing agricultural and rural goods
8) General access to preliminary training, promotion and improvement in class gaps, upgrading individual's abilities especially women
9) Upgrading health levels, creating more enjoyable and cheerful environment
10) Effective support of making and developing cooperation, and create a rural cooperative complex
11) Creating autonomous groups for responding part of financial needs of entrepreneurship projects
12) Creating small credit systems in order to present facilities like establishing rural bank
13) Creating educational consolidated centers to present educational services

And consulting in different marketing fields, caring domestic animals, handicraft industries other required skills in rural development programs must notice employment in agricultural and none-agricultural parts, population controlling, social development and urbanize the villages, reducing immigration to cities, increasing welfare level, focusing on attracting and developing new technologies in training and upbringing individual powers. With entrepreneurship development and life quality improvement in villager's lives, we can fulfill the stable rural development with a high pace moving toward a more developed and more stable country.

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Effects of methyl jasmonate vapor treatment on display quality, anthocyanin content and membrane stability index of gerbera cut flowers cv. Pink Elegance

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***Abstract*:** This study was carried out to evaluate the effect of methyl jasmonate vapor treatment on physiological and biochemical characteristics and vase life of cut flower gerbera in a completely randomized experimental design with three replications. In this experiment, cut flower stems were placed in preservative solution containing 200 mg/L 8- hydroxyquinoline sulfate and sucrose 3% and exposed to 0, 0.1, 0.2 and 0.3 µlL−1 methyl jasmonate (MeJA) and 20 µlL−1ethanol. After fumigation treatment with MeJA for 24 h, treated flowers were removed from glass chambers and placed on benches at 20 ± 2 ºC and 65 ± 5 % RH. The results indicated that different concentrations of methyl jasmonate significantly increased vase life and total postharvest longevity of flowers compared to control and ethanol. Methyl jasmonate treatments were significantly increased membrane stability index and the petal anthocyanin content compared to control and ethanol. Uptake of preservative solution under methyl jasmonate treatment was higher too. Although the relative fresh weight of flowers was higher in methyl jasmonate treatments, no statistically significant differences were found between treatments.

***Keywords*:** Gerbera, Membrane stability index, Methyl jasmonate, Vapor treatment, Vase life

I. INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus ex. Hook f.) belongs to Asteraceae family [5]. Gerbera cut flowers have short vase life under improper conditions and the major reasons for shortening of the vase life would be commonly attributed to water stress, and bacterial and fungal infection [17]. *Botrytis cinerea* Pers. is a ubiquitous fungal pathogen that causes gray mold in many fruit, vegetable and ornamental crops. Rose, gerbera and chrysanthemum are among affected cut flower species [14]. Methyl jasmonate as plant signaling molecules can modulate flowering and senescence in higher plants. In addition, jasmonates (jasmonic acid and its ester) have been revealed to be involved in direct protection against biotic stresses. These compounds are considered to act a hub role in the intracellular signaling cascades which activate inducible plant defense systems [15]. These signal molecules are involved in some signal transduction systems, which induce particular enzymes catalyzing biosynthetic reactions to form defense compounds such as polyphenols, alkaloids or pathogenesis-related (PR) proteins [18]. Methyl jasmonate has also been reported to promote vase life of several cut flowers, including freesias (*Freesia hybrida* L.), roses (*Rosa hybrida* L.) and peonies (*Paeonia lactiflora* L.) by suppressing *Botrytis cinerea* or by closing the stomata in kalanchoe (*Kalanchoe blossfeldiana* L.) and nicotiana (*Nicotiana glauca* L.) flowers. It is also shown that MeJA vapor treatment with concentration of 0.1 μlL^{-1} enhanced vase life of cut rose flower cv. 'First Red' [7].

The purpose of this study was to investigate the effect of methyl jasmonate vapor treatment on postharvest quality, physiological and biochemical characteristics of gerbera cut flowers cv. 'Pink Elegance'.

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II. MATERIAL AND METHOD

This Research was carried out in Postharvest Physiology Laboratory, Department of Horticultural Sciences, Tarbiat Modarres University to evaluate the effects of methyl jasmonate vapor treatment on ornamental quality and vase life of gerbera cut flowers cv. Pink Elegance. Flowers were harvested from commercial greenhouse (Pakdasht, Tehran, Iran) based on commercial index at early morning. Following transporting to the Laboratory, stems of cut flowers were placed in water for 1 hour to rehydration, and then stems were recut to 40 cm length. After recording the fresh weight and solution uptake, flowers were placed in preservative solution containing 200 mg/L^{-1} 8-hydroxyquinoline sulfate and 3% sucrose. In order to apply methyl jasmonate vapor, cut flowers were placed into 200 liter glass chambers for 24 hours. The desirable volume of MeJA was mixed with 20 μlL^{-1} of ethanol and poured onto filter paper inside the glass chambers to meet the required concentrations of 0, 0.1, 0.2 and 0.3 μlL^{-1} MeJA, and then the glass chambers were immediately sealed. In addition, cut flowers were treated with only 20 μlL^{-1} of ethanol as control. The postharvest characteristics were assessed daily under constant conditions: 20 ± 2 ºC and 65 ± 5% relative humidity (RH).

Vase life and Total postharvest longevity

Vase life of cut flower in considered as the duration of flower sustainability in the hands of consumers. In other words, the vase life of flower is time interval between the end of treatment of cut flowers until the flower lose their ornamental value. Vase life was determined as the number of days to 50 percent of flowers wilting [13]. Total postharvest longevity in fact express duration of transportation and treatment that expressed as days [8].

The rate of vase solution uptake

Due to the microbial growth in cut stems which blocks the vascular system, solution uptake and subsequently flower wilting are affected by stem blockage. Average solution uptake was calculated as: solution uptake ($g\ stem^{-1}d^{-1}$) = $(S_{t-1}-S_t)$; where, S_t is weight of vase solution (g) at t= days 0, 2, 4, etc., and S_{t-1} is weight of vase solution (g) on the day before [10, 13].

Relative fresh weight

Relative fresh weight (RFW) of stems was calculated as: RFW (%) = $(W_t/W_{t-0}) \times 100$; where, W_t is weight of stem (g) at t= days 0, 2, 4, etc., and W_{t-0} is weight of the same stem (g) at t=day0 [10, 13].

Anthocyanins of petals

Petals tissues (0.2 g) were ground in liquid nitrogen and 3 ml of acidified methanol (methanol and hydrochloric acid in a ratio of 99 to 1) was added to it. Then extract was centrifuged at 12000 rpm, for 20 minutes at 4^0C. Supernatants were placed overnight in the dark at 4^0C. Absorption rate was measured by spectrophotometer at wavelength of 550 nm and the amount of anthocyanin was calculated using the extinction coefficient (ε = 33000 $mol^2\ cm^{-1}$) [12].

Membrane stability index of petals

Membrane stability index of petals was determined by recording the electrical conductivity of leachates in double distilled water at 40 and 100 ^{0}C. Two similar petal disks (0.2 g) were cut to uniform size and placed in to separate test tubes containing 10 ml of double distilled water. One disk was kept at 40 ^{0}C for 30 min and the other at 100 ^{0}C in boiling water bath for 15 min and their respective electric conductivities C_1 and C_2 were measured with a conductivity meter [6].
Membrane stability index= $[1- (c_1/c_2)] \times 100$

Statistical analysis

Treatments were arranged in a repeated measures experiment, based on a completely randomized design with three replications for each treatment. Significant effects of treatments were identified by analyzing data using SPSS software version16. Mean comparisons to identify significant differences among treatment were performed using least significant difference (LSD method) and graphs were plotted using Microsoft Excel.

III. RESULTS AND DISCUSSION

Vase life and Total postharvest longevity

Effect of treatments on vase life and total postharvest longevity of cut flowers of Gerbera was significant at the 1% level (Table 1). Mean comparisons showed that the vase life resulted from 0.1, 0.2 and 0.3 μlL^{-1} MeJA were significantly increased compared to control and ethanol. Although the 0.2 μlL^{-1} MeJA treatment extended the vase life to 15.67 day, compared with the control (11.17 days) and ethanol (11days), no significant difference in vase life was found between various MeJA concentrations. The concentrations of 0.1 μlL^{-1} and 0.3 μlL^{-1} MeJA resulted in the span of vase life to 15.17 and 14.67 day, respectively (Figure 1). Treatments 0.1, 0.2 and 0.3 μlL^{-1} MeJA significantly increased total postharvest longevity compared to control and ethanol, but no statistically difference was found between various MeJA concentrations (Figure 1).

According to our data, increasing the vase life of cut gerbera under methyl jasmonate treatments could be related to the improvement of membrane stability index and reduction of lipid peroxidation. Methyl jasmonate plays an important role in the control of postharvest diseases and antimicrobial properties its causes reducing of petal wilting and increasing of vase life. Same result was reported by application of 0.1 μlL^{-1} MeJA vapor on cut flowers Freesia which improved the quality and vase life of flowers [2, 3].

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Table 1: Analysis of variance for vase life and total postharvest longevity of Gerbera cut flowers

***: Significant at P < 0.05. **: significant at P < 0.01. Ns: not significant.**

Source of variation	df	Mean squares	
		Vase life (day)	Total postharvest longevity
Methyl jasmonate	4	15.392 **	15.392 **
Error	10	1.467	1.467
Coefficient of Variation (%)	–	8.95	8.33

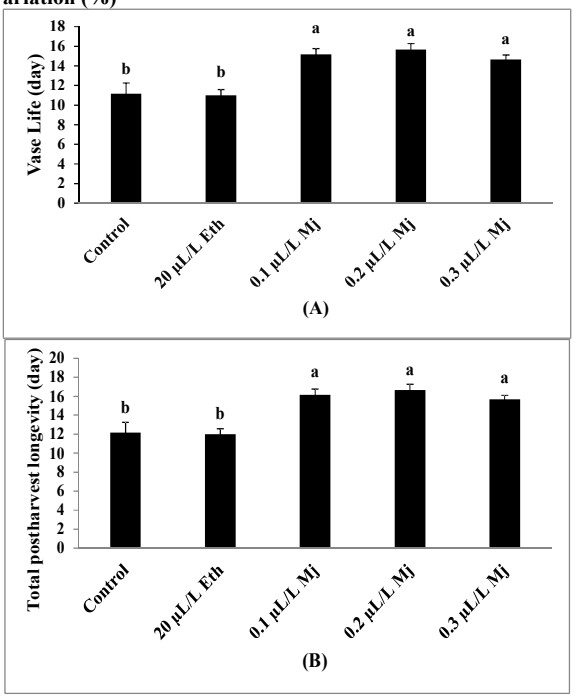

Figure 1: Effect of methyl jasmonate vapor treatment on vase life (A) and total postharvest longevity (B) of Gerbera cut flowers

Uptake of preservative solution and relative fresh weight

Comparison of means showed that during the experimental period, uptake of vase solution increased until the 6th day of experiment, then declined. In the end of experiment, the highest rate of vase solution uptake (18.897 mL) was shown by 0.2 μIL^{-1} MeJA, while the lowest rate (12.497 mL) was recorded from 20 μIL^{-1} of ethanol, (Figure 2).

The main factor for determining the display quality and vase life of cut flowers is water balance which resulted from ability of water uptake and transpiration processes [4]. When transpiration rate is higher than the water absorption, the cut flowers were faced with water deficit and exposed to flower wilting [9]. One of the reasons of flower wilting is the inability in water uptake intensified by vascular occlusion caused by bacterial growth in vascular system [10]. Meir *et al* (1998) reported that vase solution containing 200 µM methyl jasmonate was effective in controlling gray mold on 6 varieties of cut rose flowers. MJ pulsing seems to provide systemic protection against Botrytis rot by inducing resistance mechanisms in the treated cut roses and direct inhibitory effects on germination and growth of gray mold. Increasing of vase life by MJ may be due to increasing the uptake of preservative solution.

Mean comparison of duration of experiment showed the greatest relative weight on the sixth day with the 102.348 %, and the lowest in the last day (16th day), with 72.271 % and statistically significant differences was observed between days (Figure 2). Decreasing the fresh weight of cut flowers is the onset of senescence process. Old flower have little ability to absorb water and finally will face with reduction of cellular turgor [11]. Decreasing in relative fresh weight of flower is likely occurred due to reducing of preservative solution uptake and increasing of water loss [1].

Table 2: Analysis of variance for physiological characteristics of gerbera cut flowers

Source of variation	df	Mean squares	
		Uptake of preservative solution (mL)	Relative fresh weight (%)
Methyl jasmonate	4	71.726 **	53.728 ns
Error	10	9.202	53.116
Time	8	1940.064**	1671.862**
Methyl jasmonate × Time	32	3.012 ns	8.659 ns
Error (Time)	80	3.153	9.786
Coefficient of Variation (%)	–	8.10	3.34

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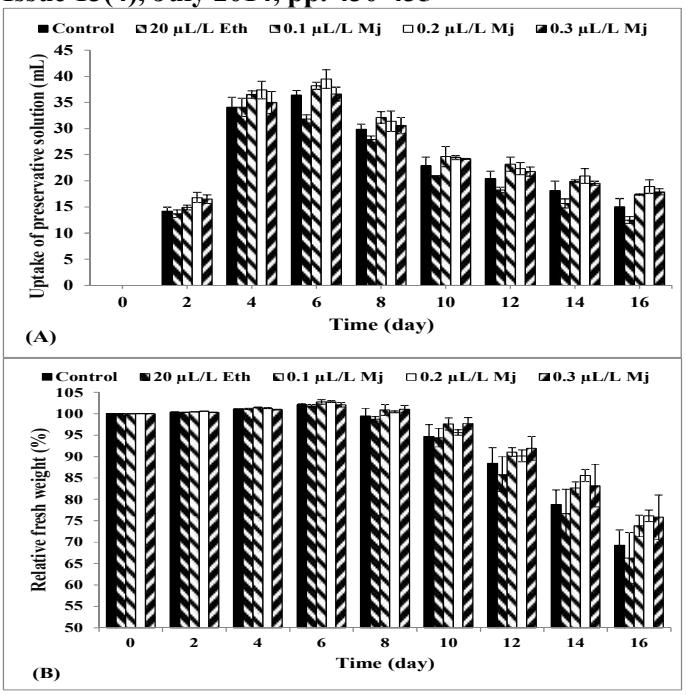

Figure 2: Effects of methyl jasmonate vapor treatment over time on the uptake of preservative solution (A) and relative fresh weight (B) of gerbera cut flowers

Membrane stability index of petals

Effects of treatments, time and interaction effects on membrane stability index were significant at 1% level (table 3). Comparison of means showed that the stability of the membrane was reduced during the experimental period. Methyl jasmonate concentrations showed higher membrane stability compared to control and ethanol, during the experimental period. Although the 0.2 µlL^{-1} MeJA had higher rate of membrane stability compared with other treatments, but no statistically significant difference was found between various MeJA concentrations (Figure 3). Reactive oxygen species tend to attack cell membranes and it is assumed that the decreasing in membrane stability is due to increasing activity of reactive oxygen species and a decreasing in the activity of antioxidant enzymes [6]. It has been reported that methyl jasmonate with increasing the activities of antioxidant enzymes and reduction of reactive oxygen species could increase the membrane stability index and shelf-life [18].

Table 3: Analysis of variance for membrane stability index of gerbera cut flowers

		Mean squares
Source of variation	**df**	**Membrane stability index (%)**
Methyl jasmonate	4	69.070 **
Error	10	1.864
Time	5	1778.565**
Methyl jasmonate × Time	20	11.815 **
Error (Time)	50	0.344
Coefficient of Variation (%)	–	0.71

*: Significant at P < 0.05. **: significant at P < 0.01. Ns: not significant.

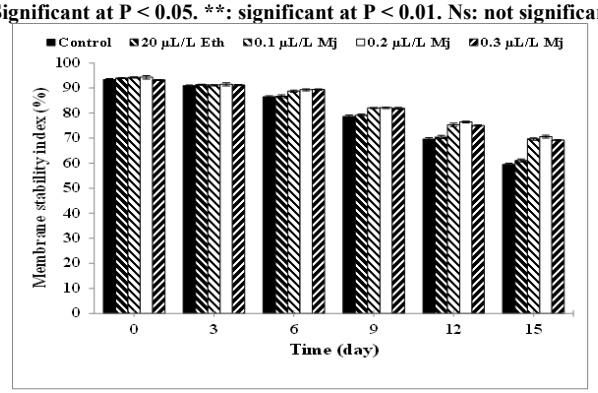

Figure 3: Effects of methyl jasmonate vapor treatment over time on membrane stability index of gerbera cut flowers

Anthocyanin content of petal

Mean comparisons showed that the anthocyanin content of petal was increased during the maintenance period. Methyl jasmonate treatments during the experimental period was significantly higher anthocyanin content than ethanol and controls. 0.2 µlL^{-1} MeJA treatment had higher rates compared with other treatments (Figure 4).

MeJA is able to activate the enzymes responsible for the biosynthesis of polyphenols, such as the phenylalanine ammonia-lyase (PAL) enzyme. The activation of PAL following postharvest application of its has been reported in many studies in fruits such as lychees, peaches, apples, plums, table grapes, strawberries with a subsequent increase of total phenols. The activation of chalcone synthase (CHS), stilbene synthase (STS), UDP glucose: flavonoid-O-transferase (UPGT), proteinase inhibitors and chitinase gene expression has also been reported in pre-harvest treatments of grapevine with MeJ. Such activations triggered the accumulation of both stilbenes and anthocyanins in cells. In a different fruit, red raspberry, the enhancement in the levels of myricetin, quercetin and kaempferol has also been reported after postharvest treatment with MeJ. Application of postharvest treatment with MeJ resulted in higher amounts of total phenols and anthocyanins in tomatoes, pomegranates, strawberries and bayberries with an increase of other flavonoids [16].

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Table 4: Analysis of variance for anthocyanin content of gerbera cut flowers

		Mean squares
Source of variation	**df**	**Anthocyanin content (µM/g FW)**
Methyl jasmonate	**4**	**162.641 ****
Error	**10**	**5.088**
Time	**2**	**2118.178****
Methyl jasmonate × Time	**8**	**27.858 ****
Error (Time)	**20**	**4.104**
Coefficient of Variation (%)	**-**	**4.39**

***: Significant at P < 0.05. **: significant at P < 0.01. Ns: not significant.**

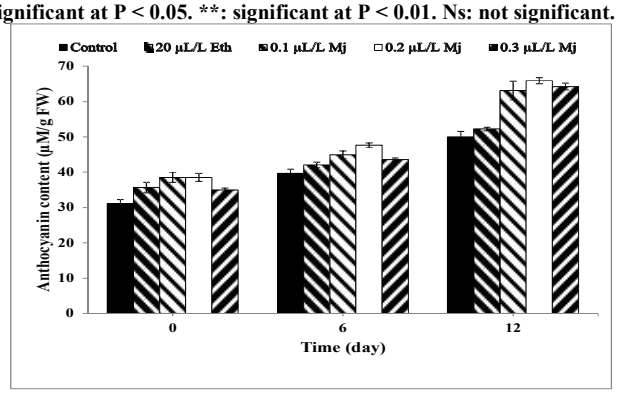

Figure 4: Effects of methyl jasmonate vapor treatment on anthocyanin content of gerbera cut flowers

IV. CONCLUSION

Methyl jasmonate vapor treatments increased the vase life and total postharvest longevity of gerbera cut flowers and improved cut flower display quality. The solution uptake, relative fresh weight and anthocyanin content were significantly affected by methyl jasmonate. Moreover, MeJA treatments enhanced membrane stability index leading to reduction ion of leakage and delaying the petal senescence.

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