# COMPARISON OF SALINITY AND DROUGHT STRESS EFFECTS ON SOME MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS IN ONION (*ALLIUM CEPA* L.) DURING EARLY GROWTH PHASE

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# Abstract

HANCI, F. and E. CEBECI, 2015. Comparison of salinity and drought stress effects on some morphological and physiological parameters in onion (*Allium cepa* 1.) during early growth phase. *Bulg. J. Agric. Sci.*, 21: 1204–1210

This study was conducted to investigate the effects of drought and salinity on four onion (*Allium cepa* L.) cultivars (Texas Early Grano, Kantartopu-3, Besirli-77 and Akgun-12) in the early plant growth phase. Seeds were germinated in peat material. After 21 days of sowing, seedlings were transferred to plastic pots (1.6 L). The plants have been grown in vermiculite by "substrate culture" technique. After ten days of transplanting, irrigation was stopped gradually for drought stress. For this aim, three different levels of irrigation were used. (D<sub>0</sub> was 90% of field capacity, D<sub>1</sub> was 70% of field capacity, and D<sub>2</sub> treatment was 40% of field capacity). In order to determination of effects of salinity on onion plants, 0. 50 mM and 125 mM NaCl were added to nutrition solution. To determine the effect of salinity and drought stress factors, tolerance indexes based on plant neck diameter (mm), leaf diameter (mm), plant length (cm), amount of leaves, leaf water potential (%) and waxy on leaves were measured as morphological parameters. At the same time, amount of proline, the concentrations of total chlorophyll ( $\mu$ g/ml), chlorophyll-a ( $\mu$ g/ml), chlorophyll-b ( $\mu$ g/ml) carotenoids ( $\mu$ g/ml) and chlorophyll-a/b were investigated as physiological parameters. Cultivars showed different responses to drought and salinity. The significant varietal differences were observed for the proline amount, chlorophyll-a, leaf water potential index and leaf amount under drought stress; and for the proline amount, chlorophyll-a under salinity stress.

Keywords: drought, salinity, abiotic stress, tolerance, proline

# Introduction

Onion (*Allium cepa* L.) is an important crop that is now cultivated globally. According to the most recent data of the United Nations Food and Agriculture Organization (FAO), worldwide onion production was approximately 83 million tons in 2012 from 4.20 million hectares. According to the FAO, Turkey produces 1.81 million tons of onions annually, which is 2.1% of world onion production, and it ranks as the 7<sup>th</sup> largest onion producer (FAO, 2011).

Although the maximum root penetration of onion is at 0.76 m, most of the roots are in the top 0.18 m of soil and only a few roots are found deeper than 0.31 m. This trait limits the amount of soil water available to the onion, especially when grown on coarse-textured soils. Most likely, irrigation water that moves

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below 0.76 m is not available to the onion crop (Drinkwater and Janes, 1955). Seed germination and emergence are critical to the survival of plants in salt-affected areas (Khan, 2002). Among crop species, different threshold tolerances (Electrical conductivities, EC) and different reduction rates of yield are seen and this indicates that there is variation in salt tolerance mechanisms (Chinnusamy and Zhu, 2005). The onion is very sensitive to EC values as low as 1.2 dSm<sup>-1</sup> and to water stress because of root system (Koriem et al., 1994; Maas, 1977). It is necessary to screen onion genotypes/varieties for salt tolerance so that improved lines can be developed (Joshi and Sawant, 2011). Few research studies have been conducted to characterize of response to drought and salt stress tolerance in onion. In a trial with several vegetable crops, Singh and Alderfer (1966) observed that soil-water stress at any growth stage leads to reduction in quality characters of onion. Dragland (1974) reported that, when compared to an unstressed control treatment, an imposed 3-week-long drought early in the season reduced onion yield more than when the 3-week drought was imposed near the end of the growing season. Jafarzadeh and Aliasgharzad (2001), Mangal et al. (1991), Stino et al. (1972), Wannamaker and Pike (1987) have investigated the influence of salinity on seed germination, growth, flavor, and yield attributes in onion. Our previous study has shown that there are significant differences among onion cultivars for salinity tolerance (Hanci et al., 2012).

The purpose of this study was to compare the effects of drought and salt stress on four onion cultivars using both morphological and physiological parameters in early growth phase and determine the differences of cultivars.

#### **Materials and Methods**

The study was conducted between 2012 and 2013. The morphological and physiological changes of several local onion cultivars in Turkey were tested at different irrigation and salinity levels. 'Kantartopu-3' (Medium/large size, middle/ long day, dark yellow skin), 'Akgün-12' (Small/medium size, middle-day, dark vellow skin) and 'Besirli-77' (Small/medium size, middle-long day, dark red skin) seeds were obtained from the Atatürk Central Horticultural Research Institute in Yalova. 'Texas Early Grano' seeds were provided by the commercial seeds sales office. Seeds of onion were sown in viols which filled with perlite/peat (1:1) mixture. Three weeks old onion seedlings were then transplanted into 1.6 liter pots. These pots were filled with 1.5 liter of vermiculite. The transplanted onion seedlings were watered a two-days period with Hoagland's solution (Hoagland and Arnon, 1950) before initiating water treatments in order to improve root development. The experiments were conducted in the laboratories of the Department of Vegetable Breeding and Tissue Culture, and Greenhouse at the Atatürk Central Horticultural Research Institute.

#### Drought Experiment

Six pots with one seedling each were randomly assigned to each of the three levels of water until end of the experiment. Drought treatments were started 10 days after transplanting. The amount of water to be added was determined based on the percentage of pot water capacity. Pot (included vermiculite) water content by gravimetric test was observed in the end of drought stress application ( $D_0$  was 90% of field capacity,  $D_1$  was 70% of field capacity, and  $D_2$  treatment was 40% of field capacity). The 70% field (pot) capacity was chosen as drought starting point because Sanders (1997) reported that this soil moisture rate is minimum point for onion. Application of drought stress was designed according to Djekoun and Planchon (1991) method with little modification. Djekoun and Planchon applied stress by stopping watering for 4, 8 and 10 days, whereas we formed drought by stop watering for five days.  $D_0 = \text{control} (90\% \text{ FC})$ , the plant watering at the twoday period.  $D_1$ : (70%) beginning of drought stress carried by stops watering to plants during five days.  $D_2$ : (40%) drought stress, carried by stop watering to plants during ten days.

#### Salinity Experiment

Six pots with one seedling each were randomly assigned to each of the three levels of salinity until end of the experiment. Salt treatments were started with the drought treatments. Salinity level in the nutrition solution was increased by steps of 25 mM per irrigation until the final NaCl concentration was reached 50 Mm ( $S_1$ ) and 125 Mm ( $S_2$ ). Nutrition solution without NaCl served as the control ( $S_0$ ).

#### Measurements

For determination of tolerance differences between cultivars using morphological parameters, salt/drought tolerance indexes were calculated based on plant neck diameter (mm), plant lenght (cm), leaf number (amount), leaf diameter (mm) after five weeks of transplanting. Also waxiness on leaves was measured according to the International Union for the Protection of New Varieties of Plants (UPOV). On the other hand, assessments of total chlorophyll (µg/ml), chlorophyll-a (µg/ml), chlorophyll-b (µg/ml), chlorophyll a/b, proline (µmol/g), leaf water potential (%) and carotenoids content  $(\mu g/ml)$  were performed as physiological parameters. Chlorophyll and carotenoids content was determined in 80% acetone extract. After centrifugation (14.000 rpm, 20 min) the absorbance was read spectrophotometrically at 663, 652, 646 and 470 nm. The concentrations were calculated according to Lichtenthaler and Welburn (1983) method. Proline was extracted from a sample of 0.5 g fresh leaf material samples in 3% (w/v) aqueous sulphosalycylic acid and estimated using the ninhydrin reagent according to the method of Bates et al. (1973). The absorbance of fraction with toluene aspired from liquid phase was read at a wave length of 520 nm. Proline concentration was determined using a calibration curve and expressed as µmol proline g<sup>-1</sup> fresh weight. For comparison of multiple means, one-way ANOVA and the LSMeans Differences Student's test were used. Significant difference in statistical tests was set at P < 0.01.

## Results

The results were evaluated under two separate groups as physiological and morphological parameters for both drought

and salinity. The results of variance analyses for the measured characters were presented in Table 1. According to results of drought experiment, proline amount, chlorophyll-a, tolerance index for leaf water potential and leaf amount were significant for three factors (cultivar, drought and their interaction) while total chlorophyll, chlorophyll-b, carotenoids, tolerance index for length, leaf diameter, neck diameter and waxiness were significant for the only drought. In the salinity experiment, proline amount, chlorophyll-a and tolerance index for leaf diameter were significant for three factors (cultivar, salinity and their interaction). Total chlorophyll, chlorophyll a/b, carotenoids, tolerance index for length, neck diameter, leaf amount and waxiness were significant for the only salinity (Table 1).

Generally, all physiological parameters were reduced by increased both drought and salinity level except for degree of leaf waxiness and proline amount. Results were summarized on the Tables 2 and 3. The change of proline and chlorophyll-a amount were significant for three factors (cultivar, drought/salinity and their interaction) (P < 0.01). Proline increased significantly under both salinity and drought stress in comparison with control in all cultivars except for Besirli-77 cultivar under drought stress. The maximum mean proline content was recorded at D<sub>2</sub> treatment in cultivar Texas Early Grano (12.89 µmol/g). The salinity caused stronger increase of proline levels than drought. For example, under drought stress, the proline amount was increased from 4.16 µmol/g to 5.03 µmol/g which was 1.21 fold higher than control while under salinity stress; it was increased from 4.16 µmol/g to 7.28 umol/g which was 1.75 fold higher than control in Kantartopu-3 cultivar. Chlorophyll-a decreased significantly under both salinity and drought stress in comparison with control in all cultivars except for Texas Early Grano cultivar under drought stress. Unlike proline amount, chlorophyll-a level was more decreased under drought conditions than salinity. For example, under drought stress, in Kantartopu-3 cultivar, the chlorophyll-a amount was decreased from 6.23 µmol/g to  $0.97 \,\mu$ mol/g which was 0.15 fold lower than control but it was decreased from 6.23 µmol/g to 5.23 µmol/g which was 0.84 fold lower than control in salinity experiment. The change of leaf water potential (LWP) was effected for three factors (cultivar, stress and their interaction) only under drought stress. Leaf water potential (LWP) percentages were reduced by increased drought level. That effect was more pronounced in 'Akgun-12' and 'Besirli-77' than in other cultivars. For example, in the D<sub>2</sub> treatment, leaf water potential was decreased from 91.45% to 84.68% which was 1.31 fold lower than control in "Texas Early Grano".

All morphological parameters were reduced by increased both drought and salinity level (Table 4). Under the drought conditions, only tolerance index for the amount of leaves  $(LA_{TI})$  was significantly affected from three factors (cultivar,

			Expe	riment 1: F	Ratios	Experiment 2: F Ratios			
	C. Total	Error	Cultivars	Drought	Cultivars x Drought	Cultivars	Salinity	Cultivars x Salinity	
DF	47	36	3	2	6	3	2	6	
Р			73.66*	173.48*	41.08*	23.31*	57.11*	163.16*	
C <sub>T</sub>			2.01ns	4.96*	2.07 ns	3.59*	5.46*	0.73 ns	
			3.29*	8.44*	4.53*	8.55*	36.47*	6.22*	
			1.34 ns	3.92*	0.88 ns	3.57*	0.72 ns	1.77 ns	
C <sub>a/b</sub>			0.26 ns	0.68 ns	0.74 ns	0.98 ns	8.07*	2.02 ns	
Lwp			15.32*	50.99*	7.67*	0.88 ns	1.15 ns	0.39 ns	
Crt			0.67 ns	3.56*	0.91 ns	1.59 ns	3.48*	0.49 ns	
L <sub>II</sub>			1.93 ns	50.46*	0.75 ns	1.57 ns	59.31*	1.11 ns	
LDM <sub>TI</sub>			1.55 ns	17.48*	0.51 ns	7.19*	60.47*	4.14*	
NDM <sub>TI</sub>			1.08 ns	36.60*	0.64 ns	1.48 ns	48.65*	2.46 ns	
			7.05*	51.11*	2.40*	4.15*	24.62*	1.60 ns	
Wxn.			15.09*	90.13*	2.30 ns	5.42*	18.11*	0.39 ns	

Table 1A two-way variance analysis

\*Significant (p < 0.01), ns: Non significant F: Freedom, DF: Degree of freedom, C. Total: Corrected Total,  $C_a$ : Chlorophyll a,  $C_b$ : Chlorophyll b,  $C_T$ : Total Chlorophyll,  $C_{ab}$ : Chlorophyll a/b Crt: Carotenoids, P: Proline, LWP: Leaf Water Potential,  $L_{TI}$  = Tolerance index for plant lenght, LDM<sub>TI</sub> = Tolerance index for leaves diameter, NDM<sub>TI</sub> = Tolerance index for neck diameter, LA<sub>TI</sub> = Tolerance index for amount of leaves Wxn.: Waxiness

## Table 2

The effect	t of drought	and salinity on	chlorophyll	amounts
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Clt.	Lv.	C	n ía	0	n ´h	C <sub>T</sub>		$C_a/C_b$	
		D	S	D	S	D	S	D	S
	0	6.23 bc	6.23 ab	4.64	4.64	12.18	12.18	1.44	1.44
Knt.	1	5.58 bcd	6.26 ab	4.38	4.39	11.37	11.65	1.29	1.53
	2	5.25 cde	0.97 d	4.56	6.15	11.43	10.26	1.2	0.16
	Avr.	5.68	4.49	4.52	5.06 a	11.66	11.36	1.3	1.04
	0	7.67 a	7.67 a	5.21	5.21	14.62	14.62	1.59	1.59
Alex	1	5.49 bcd	6.11 b	4.34	4.81	11.29	12.4	1.27	1.35
Akg.	2	4.48 de	3.98 c	3.68	4.18	9.09	9.23	1.22	1
	Avr.	5.88	5.92	4.41	4.73 a	11.66	12.08	1.4	1.31
Dan	0	6.53 ab	6.53 ab	4.8	4.8	12.93	12.93	1.46	1.46
	1	4.71 de	3.48 c	3.9	4.13	9.67	8.67	1.21	0.88
Bsr.	2	5.01 cde	3.63 c	3.82	4.1	9.95	8.84	1.31	0.91
	Avr.	5.42	4.55	4.17	4.38 ab	10.85	10.14	1.3	1.08
TEG	0	4.15 e	4.15 c	4.06	4.06	9.31	9.31	1.08	1.08
	1	5.53 bcd	3.93 c	4.1	3.82	10.81	8.73	1.35	1.09
	2	4.76 de	3.14 c	3.71	3.7	9.53	7.81	1.28	0.89
	Avr.	4.81	3.74	3.95	3.86 b	9.88	8.62	1.2	1.02
	0	6.15	6.15	4.68 a	4.68	12.26 a	12.26 a	1.39	1.39 a
Mean**	1	5.33	4.94	4.18 ab	4.29	10.79 ab	10.36 ab	1.28	1.21 a
1. r	2	4.88	2.93	3.94 b	4.56	10.00 b	9.04 b	1.25	0.74 b

\*Means within a column that have a different small letter are significantly different from each other (P < 0.01). Lettering was made according to results of variance analyses \*\* Means average of each treatment. Clt= Cultivar, Lv=Stress level, Knt= 'Kantartopu-3', Akg='Akgün-12',Bsr=Besirli-77, TEG=Texas Early Grano. D=Drought. S= Salinity.  $C_a$ = Chlorophyll-a,  $C_b$ = Chlorophyll-b,  $C_T$ = Total Chlorophyll, Avr=Average of each cultivar.

## Table 3

## The effect of drought and salinity on carotenoid, proline and leaves water potential

Clt.	Lv.	C	rt	I	)	LWP		
		D	S	D	S	D	S	
	0	1.14	1.14	4.16 d	4.16 ef	95.67 a	95.67	
Knt.	1	0.7	1.09	3.33 ef	6.00 bcd	95.34 a	92.44	
	2	0.57	0.54	5.03 c	7.28 b	93.64 ab	91.93	
	Avr.	0.8	0.92	4.17	5.81	94.88	93.35	
	0	1.49	1.49	7.63 b	7.63 b	95.84 a	95.84	
A 1	1	0.84	1.13	3.11 fg	6.16 bc	88.04 cd	95.26	
Akg.	2	0.68	0.65	7.71 b	8.40 ab	78.68 e	94.45	
	Avr.	1	1.09	6.15	6.06	87.52	95.18	
D	0	1.24	1.24	3.57 def	3.57 ef	95.40 a	95.4	
	1	0.66	0.55	2.55 gh	2.92 f	89.23 bcd	93.64	
Bsr.	2	0.79	0.64	3.14 efg	4.89 cde	73.10 f	88.67	
	Avr.	0.9	0.81	3.09	3.79	85.91	92.57	
TEG	0	0.63	0.63	3.86 de	3.86 ef	91.45 abc	91.45	
	1	0.8	0.65	2.10 h	6.61 b	90.92 abc	92.1	
	2	0.79	0.44	8.80 a	12.89 a	84.68 d	91.8	
	Avr.	0.74	0.57	4.92	7.78	89.02	91.78	
	0	1.13 a	1.13 a	4.81	4.81	94.59	94.59	
Mean**	1	0.75 b	0.85 ab	2.77	5.42	90.88	93.36	
	2	0.71 b	0.57 b	6.17	7.37	82.52	91.71	

\*Means within a column that have a different small letter are significantly different from each other (P < 0.01).Lettering was made according to results of variance analyses \*\* Means average of each treatment. Clt= Cultivar, Lv=Stress level, Knt= 'Kantartopu-3', Akg='Akgün-12',Bsr=Besirli-77, TEG=Texas Early Grano. D=Drought. S= Salinity. Avr=Average of each cultivar. Crt: Carotenoids, P: Proline, LWP: Leaf Water Potential

Clt.	Lv.		Tolerance Index								Wxn	
CII.	LV.	L <sub>11</sub> (%)		LDM <sub>TI</sub> (%)		$NDM_{TI}(\%)$		$LA_{TI}(\%)$		VV XII		
		D	S	D	S	D	S	D	S	D	S	
Knt.	0	100	100	100	100 a	100	100 a	100 a	100	1.5	1.5	
	1	82.63	83.44	71.21	98.65 a	73.86	98.67 a	90.00 ab	90	3	2.5	
	2	65.94	75.75	63.25	61.60 de	60.94	61.88 b	85.00 bc	78.75	6.5	3.5	
	Avr.	82.85	86.4	78.15	86.75	78.26	86.85	91.67	89.58	3.67	2.5	
A 1- ~	0	100	100	100	100 a	100	100 a	100	100	3	3	
Akg.	1	73.36	83.41	80.69	88.86 ab	74.95	68.77 b	86.67 b	72.5	4.5	3.5	
	2	41.1	60.54	64.12	82.55 bc	43.09	65.18 b	69.17 de	68.34	7	4.5	
	Avr.	71.48	81.32	81.6	90.47	72.68	77.99	85.28	80.28	4.83	3.67	
D	0	100	100	100	100 a	100	100 a	100	100	3	3	
	1	71.47	81.87	55.91	73.08 cd	78.04	97.40 a	73.07 cde	74.11	4	3.5	
Bsr.	2	43.59	56.41	35.34	52.77 e	56.83	58.90 b	51.34 f	46.73	6.5	4.5	
	Avr.	71.69	79.43	63.75	75.28	78.29	85.43	74.8	73.61	4.5	3.67	
	0	100	100	100	100 a	100	100 a	100	100	1.5	1.5	
TEC	1	66.33	75.57	80.18	99.97 a	69.3	85.59 a	81.25 bcd	93.75	3	3	
TEG	2	47.16	60.84	54.01	67.26 d	33.83	61.66 b	62.5 ef	75	4	4	
	Avr.	71.16	78.8	78.06	89.08	67.71	82.42	81.25	89.58	2.83	2.83	
	0	100 a	100 a	100 a	100	100 a	100	100	100 a	2.25 c	2.25 c	
Mean**	1	73.44 b	81.07 b	71.99 b	90.14	74.04 b	87.61	82.75	82.59 b	3.63 b	3.13 b	
	2	49.45 c	63.38 c	54.18 c	66.05	46.67 c	61.91	67	67.20 c	6.00 a	4.13 a	

Table 4
The effect of drought and salinity on morphological parameters in onion cultivars

\*Means within a column that have a different small letter are significantly different from each other (P < 0.01).\*\* Means average of each treatment. Clt= Cultivar, Lv=Stress level,  $L_{TI}$  = Tolerance index for plant lenght, LDM<sub>TI</sub> = Tolerance index for leaf diameter, NDM<sub>TI</sub> = Tolerance index for neck diameter, LA<sub>TI</sub> = Tolerance index for amount of leaves, Wxn=Leaf waxiness, Knt= 'Kantartopu-3', Akg='Akgün-12',Bsr=Besirli-77, TEG=Texas Early Grano. D=Drought.S= Salinity. Avr=Average of each cultivar.

drought and their interaction) (P < 0.01). Tolerance index for the amount of leaves (LA<sub>TI</sub>) on per plant decreased under drought stress in comparison with control in all cultivars. The maximum tolerance index at D<sub>2</sub> treatment was recorded in "Kantartopu-3" cultivar (85%). Leaf and neck diameter tolerance indexes (LDM<sub>TI</sub> and NDM<sub>TI</sub>) were significant for tree factors (cultivar, salinity and their interaction). Akgun-12 cultivar was more tolerant to salinity than other cultivars based on the results of leaf and neck diameter tolerance indexes (LDM<sub>TI</sub> and NDM<sub>TI</sub>). The LDM<sub>TI</sub> and NDM<sub>TI</sub> were recorded as 82.55% and 65.18% respectively in this cultivar whereas same parameters were measured as 52.77% and 58.90% respectively in Besirli-77 under the S<sub>2</sub> treatment.

#### Discussion

Plants may be affected by drought at any time of life, but certain stage such as germination and seedling growth are critical (Pesarakli, 1999). The morphological symptoms were easily observed 2-3 weeks after stress applications. Both drought and salinity stress affected all morphological parameters. But, varietal differences were observed for leaf diameter (under salinity), neck diameter (under salinity) and amount of leaves (under drought).

In response to drought and salinity stress, many plant species accumulate high levels of proline, which is thought to function in stress adaptation (Adams and Frank, 1980). Under drought conditions, an increase of proline content in different species was reported by Zgallaiel et al. (2005), Vendruscoloet et al. (2007), Tatar and Gevrek (2008). Also elevated proline content in response to salinity has been reported in Goudarzi and Paknivat (2008), El-Baz et al. (2003), and Sidari et al. (2008). The results of our study are in agreement with these reports. Proline increased significantly under both salinity and drought stress in comparison with control in all cultivars except for Besirli-77 cultivar under drought stress. The varietal differences were observed for proline changes under two stress factors. Drought and salinity stress imposed significantly decreased chlorophyll-a content. For this parameter, cultivars showed different responses to drought and salinity. Under the drought conditions, ratio of C<sub>2</sub>/C<sub>b</sub> did not affected significantly. This result can explained by the balance mechanism between C<sub>a</sub> and C<sub>b</sub>. The lack of effects on the chlorophyll-b and chlorophyll-a/b ratio indicates that chlorophyll-a is more sensitive to drought and salinity than chlorophyll-b (Mafakheri et al., 2010)

Significant differences were obtained for the two stresses regarding some parameters. For example, salinity had more severe effects on proline accumulation than drought. Whereas chlorophyll-a level was less affected under drought conditions than under salinity. High drought levels had a greater effect than high salinity levels on leaf waxiness.

## Conclusion

In conclusion, drought and salinity had a great effect on different parameters. Responses of onion cultivars were different for different parameters. The proline amount, chlorophyll-a, leaf water potential and leaf amount can be regarded as a useful tool for comparison of onion cultivars/genotypes under drought stress. The proline amount, chlorophyll-a and leaf diameter can serve as useful indicators of salt tolerance. Akgün-12 appeared more tolerant to salt and drought stress than the other cultivars. This result is agreement with our previously report (Hanci et al., 2012). This work was undertaken to compare the effects of salinity and drought stress on various physiological and morphological parameters in seedlings of onion. These results may be useful for further research and breeding of new onion cultivars for drought or salinity tolerance.

#### Acknowledgements

The authors thank Yıldız DAŞGAN from the Çukurova University, Turkey for technical advice.

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Received May, 26, 2015; accepted for printing October, 5, 2015