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Influence of different sowing date and planting pattern and N rate on buckwheat yield and its quality

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Abstract

Crop management can significantly influence buckwheat yield, rutine, starch and protein content. A field trial was conducted to determine its effect in detail regarding sowing date, planting pattern and N rate during 2010-2011 in Agricultural Research Institute of Arak, Iran. Planting treatments as main plot was implemented at two levels including the mounds with the width of 50 cm associated with two planting rows with distance intervals of 20 cm (P_1) and those with the width of 60 cm along with three planting rows which are of the distance intervals of 15 cm (P_2). Sowing date was regarded as sub-plot with four different factors including 20th June (D_1), 5th July (D_2), 20th July (D_3) and 5th August (D_4), while N considered as sub-sub-plot at four different rates at 0 (control) (N_1), 50 (N_2), 100 (N_3) and 150 kg ha^{-1} (N_4), respectively. Results showed that the interaction of planting date, N rate and planting pattern significant for all the studied properties so that The highest yield of 2457 kg ha^{-1}, starch content (%) as 51.69%, protein content (%) as 15.24% and leaf, stem and flower rutine content (%) as 1.01, 0.32 and 1.36% and third and fourth sowing dates, 100 kg nitrogen and planting pattern P_2 can be generally suitable for the qualitative and quantitative yield and accumulation of rutin.

Key words: Buckwheat, sowing date, crop management, agronomic performance.

Introduction

Considering all the fact that buckwheat is of a variety of medical, industrial and food applications and in our country and some other ones (Bonafaccia et al., 2003; Alvarez et al., 2010), it has not been seriously cultivated, this plant must be used as a new plant and it should be extensively applied in multiple planting systems (summer planting) for commercial goals through producing seeds while its nutritional value is more than grain and it can be regarded as a rich source of high quality protein, amino acid necessary for lysine, high starch content, minerals and vitamins for different applications involving cake flour, frumenty and soup and improving the optimal rutin content as a secondary metabolite having effective medical features concerning our country's climatic conditions (Ikeda et al., 1994 ; Hozova et al., 2007; Christa and Soral- Smietana, 2008). Sowing date as a fundamental element for production is of considerable importance due to its significant effects on plant genotype for exploiting its maximum production potential. Thus, the most appropriate sowing date leads to obtain the maximum yield as compared to the other sowing dates. One of the important factors influencing the selection of sowing date may be temperature in addition to this plant's summer planting. Accordingly, Hu (2003) conducted an experiment with respect to summer planting of buckwheat. Determination of sowing date aims to find the appropriate planting time of cultivar or a group of similar ones so that the existing set of environmental factors can be suitable for the plant germination, establishment and survival (Hore and Rathi, 2002). Appropriate sowing date may be introduced as one of effective and important factors to achieve the suitable yield of crop. According to the experiments done by Hu (2003) concerning Buckwheat summer planting, one of the most influential elements for choosing the best sowing date is temperature. It has been reported that the best sowing date of buckwheat is more likely to be late May having the yield of 2059 kg/ha with regard to late April, early and late May, early and late June in China (Lee et al., 2001). Best sowing date of buckwheat has been suggested as mid-May to July in Western Europe (Bernath, 2000). Various sowing dates in various climatic conditions influence the growth period composition of the plant effective material and yield (Omidbeigi, 2005). It has been reported that rutin content (%) in sowing dates after summer is half of that obtained for sowing dates of summer (Li et al., 2008). Morishita et al. (1995) reported that temperature is the most important environmental factors which affect flavonoid accumulation and rutin of buckwheat (Morishita et al., 1995). Many researchers have attributed the interactions of sowing date and planting pattern to suitable bush distribution and their in order to sustain the soil humidity content(Shamsi , 2009). Changes of plants, sowing patterns with constant density can alter the conditions for light absorption concerning plant biomass. As we get closer to square and rectangular patterns, lack of light uniformity will be reduced in vegetative areas and consequently, competition for absorbing light and producing assimilates of leaves and stem may be decreased. As a result, higher material production influences the qualitative and quantitative yields. In this regard, it has been reported that the interactions of planting pattern and nitrogen enhance the nitrogen consumption in relation to the effects of nitrogen on the leaf area development and bush distribution with the same intervals and consequently, the plant yield and other properties are improved (Marois et al., 2004; Ali et al., 2012).

One of plant's basic elements is nitrogen. Buckwheat (*Fagopyrum esculentum*) response to nitrogen depends on primary nitrogen content of the soil, climatic conditions and nitrogen additives as well as its addition time. Results given by an experiment performed in Poland in 1995 on the effects of nitrogen levels on yield using 30 kg ha^{-1} nitrogen showed that the amount of 30 kg nitrogen resulted in the increase of grain yield but higher levels reduced it (Noworolink, 1995). Another research done in India reported that 50 kg nitrogen, 20 kg phosphorus and 40 kg potassium increased the buckwheat yield (Phogat and Sharma, 2000). While studying the effects of five cultivars on yield in Italy, the highest yield as 2379 kg ha^{-1} has been attributed to the cultivar of La Harpe (Brunori et al., 2005). Li and Kawiatkowski achieved the highest yield as 2500 kg ha for buckwheat. After that, the highest and the lowest yields were computed as 1890 and 821 kg ha^{-1}, respectively (Li et al., 2004; Kawiatkowski et al., 2004). The highest grain yield and rutin content have been suggested as 2000 kg and 1.21%, respectively (Chang et al., 2003). Average buckwheat yield has been suggested as 1.787 ton ha^{-1} (FAO, 2011). Researchers stated that the increase in nitrogen can enhance biomass, leaf area, number of branches and quantitative and qualitative grain yields (Noworolink, 1995). Several elements' effects such as plant density and nitrogen fertilizer have been observed on protein increase (Chai et al., 1998; Zhang et al., 1998 and 2001; Feng et al., 2003). Some researchers have proposed that as the grain weight and yield are decreased under the heat stress impacts, carbohydrates will be reduced due to the shortening of grain filling period and protein content may be enhanced as compared to the carbohydrates (Tahir et al., 2006; Subedi et al., 2007). Grain protein content has a considerable correlation with nitrogen used by soil and it can confirm the necessity of nitrogen fertilizer according to the plants' needs (Feng et al., 2003; Chai et al., 1998; Zhang et al., 2001). Buckwheat protein content has been estimated as 10.6-15.5%. Average protein content of buckwheat has been given as 12-18.9% (Stempinska and Soral-Smietana, 2006). In addition, Lu et al. (2013) presented the average protein content of buckwheat as 10.2-17.9%. In an experiment done on buckwheat with two sowing dates involving 20^{th} and 25^{th} June, the highest number of plant branches and the highest yield and weight of thousand grains were obtained for the sowing date of 25^{th} June using 60 kg ha^{-1} nitrogen (Wangberth et al., 1996). Chlopicka (2008) has introduced rutin as the most important flavonoid in buckwheat estimated as 4-6%. Another experiment on buckwheat rutin content (%) was performed while examining different sowing dates and it was presented that rutin content (%) for sowing dates after summer was half of that given for summer planting dates (Mao et al., 2003). According to the experiments done in Berlin, Germany, the highest rutin content (%) of buckwheat was achieved for 45 days after cultivation. Also, high levels of nitrogen fertilizer led to the decrease in rutin content and the increase in biomass (Hagles et al., 1995). Baumgertel et al. (2010) reported the highest rutin content for 58 days after cultivation. Most rutin content of buckwheat was proposed for the flower (Dadakova and Kalinova, 2010). Investigating buckwheat characteristics in seven sowing dates in Karaj, Iran represented the best sowing date as 25^{th} June with the highest number of plant branches, most dry matter and rutin content (%) (Omidbeig and Mastro, 2004). This experiment has been conducted to study the effects of sowing date, planting pattern and nitrogen levels on the quantitative and qualitative yields as well as rutin content of buckwheat regarding climatic conditions of Arak for developing its cultivation for the first time.

Results and Discussion

Grain yield

Triple interaction of treatments indicates the highest yield was given as 2457 kg ha^{-1} for the treatment of $P_2D_3N_3$ (Table 3). The most important characteristic studied in each experiment is the grain yield which in fact involves nearly all the conclusions since the yield components may be finally related to this factor. Like the other plants, final yield of Buckwheat will be influenced by a variety of environmental and internal factors. Plants' yield in high temperature (summer) depends on all these elements, especially the determination of suitable sowing date in order to specify carbon source and decrease the seeds' maturity period rather than the maturity speed. Seemingly, it can be assumed that such conditions may occur for the plant during the summer planting. Changing the appropriate sowing date leads to the decrease in the desired yield and the control of late sowing date is more difficult than the early one (Tobares et al., 2002). Therefore, higher grain yield concerning the third sowing date can be attributed to better vegetative growth, canopy expansion and as a result, more suitable use of solar radiation and more photosynthesis during the vegetative, flowering, pollination and seed filling stages (maturity period) followed by more stored assimilates to be retransferred in seed filling stage. Heat stress due to the increase of temperature and soil evaporation and the synchrony of flowering stage and heat stress as well as flower falling and abortion are the major reasons of yield reduction during first and second sowing dates. At fourth sowing date which coincided with the shortening of day, number of generative units is considerably decreased so that all the properties (except one thousand grain weight) were reduced in comparison with the third one. This falling trend could not compensate one thousand grain weight and reduced the grain yield. These results have been in conformity with those reported by Board et al. and Egli et al. (1999; 2000). Hore and Rati (2002) have specified August as the best sowing month in India. According to the given results, although the yield is decreased in summer, it will be an acceptable one related to the plant ability for confronting the environmental stresses through the enhancement of stored materials retransfer to the grains and the interactions of sowing date and planting pattern along with the important roles of bush distribution in order to sustain the soil humidity content; thus, considerable yield reduction can be prevented (Fig. 1). Hu and Liu (2003-2002) reported August and July as the best sowing months in summer that confirmed the given results. Chinese researchers have reported the buckwheat yield as 2500 kg ha^{-1} (Kwiatkowki, 2004; Liu et al., 2003).Evaluating the yield of 5 cultivars in Italy, the highest yield computed as 2379 kg was attributed to La Harpe (Brurnori et al., 2005). The highest grain yield has been reported as 2699 kg ha^{-1} for VL-7 (Hore and Rati, 2002). Chang et al. (2003) proposed the lowest and highest yield rates given as 821 and 1890 kg, respectively. Mao et al. (2003) have presented the highest grain yield of buckwheat as 2000 kg. Among late April, early

Table 1. Selected physico-chemical properties of soil.

Parameter	Unit	Unamended soil
pH	-	7.9
EC	dS m^{-1}	1.28
Sand	%	30
Silt	%	35
Clay	%	35
Organic carbon	%	0.6
Total P	%	0.01
$CaCO_3$	%	10

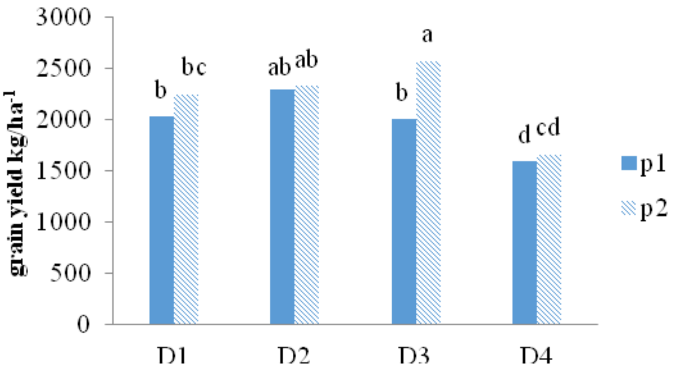

Fig1. Interaction of planting pattern and sowing date of grain yield.

May, late May, early June and late June, the best sowing date was shown as late May with the yield of 2059 kg ha^{-1} (Lazanayi and Laszlo, 2009). Stanislawa and Kvzysztof (1990) have reported 2001 kg of yield in Poland in a 2-year study of quantitative features of buckwheat. Kalinova (2009) presented the yield of 1500 kg ha^{-1} for the buckwheat. The highest buckwheat yield was proposed as 2626 kg ha^{-1} in Serbia (Popovic et al., 2014). Given that increasing the amount of nitrogen fertilizer stimulates the vegetative growth and decreases the generative period in summer and temperature effects accelerate the plant activities (the reduction of vegetative and reproductive periods), the interactions of nitrogen fertilizer, sowing date, planting pattern and plant growth type enable the plant to achieve the suitable leaf area index using 100 kg N ha^{-1} and the grains receive more shares of photosynthetic materials. Plant cannot obtain the favorable LAI, more photosynthesis and as a result, biomass production before reaching the maturity of grains in the treatments of 0 and 50 kg nitrogen. Plant relying on its poor vigor will not be successful for the retransfer of the stored materials and the yield is highly reduced.(Fig. 2)

Effects of nitrogen on the buckwheat properties and the other plants have been discussed and the findings are in conformity with those presented in this paper. Phogat and Sharma (2002) declared that using 50 kg N, 20 kg P and 40 kg K ha^{-1} can increase the buckwheat yield. Examining different densities of cultivation and nitrogen, it has been indicated that the density of 33.3 bushes with 100 kg nitrogen had the highest yield (Omidbeigi and Zakizadeh, 2002). In an intense planting pattern (non-uniform) and high densities, total yield is usually reduced due to low leaf area index and the delayed complete establishment of vegetation leading to low use of environmental resources, especially solar radiation. Considering the fixed density in the planting pattern of P_2, the bush distribution on the mounds with the width of 60 cm in 3 rows with the distance intervals of 15 cm can decrease the internal species contest and enhance the share of each plant to absorb the light, water and nutrition from the roots as compared to the mounds with the width of 50 cm in 2 rows with the distance intervals of 20 cm. In such circumstances, each bush uses more sunlight and available sources and the roots' mass is increased because of the access to wider space and the effective consumption of nutrient. Also, it seems that cold microclimatological conditions inside the canopy in the planting pattern of P_2 highly preserve the humidity content of soil leading to more use of nitrogen and show its effects on the yield (Cheema et al., 2002; Zhou et al., 2011). Seong et al, (1998) showed the grain yields of 2.48, 2.85 and 3.21 T ha^{-1} through the density treatments involving the row intervals of 30, 40 and 50 cm. Differences of grain yields in a variety of planting patterns are relevant to the different reactions of bushes while changing the available space and contest rate in order to achieve the desired resources (Hagels et al., 1995). When plants are cultivated in the rows, their internal contest will be increased due to the plant morphological changes and increase of resources' number. Most researchers believe that when the distance of planting rows is decreased, maximum grain yield will be obtained (Andrade and Calvino, 2002; Ozer, 2003). Bavec et al. (2005) conducted a 2-year study of 2 cultivars, the accumulation and growth period of buckwheat and observed the meaningful effect of year on the grain yield. Brunori et al. (2005) examined 5 cultivars of buckwheat with 300 bushes (m^{-2}), the planting pattern having the 25 cm rows and 15 kg N and found a negative correlation between the yield and the harvest index. Table 4 shows the correlation coefficients of studied traits and demonstrates that the grain yield has positive and significant correlations with leaf rutin content (0.26%), stem (0.28), seed (0.48), flower rutin content (0.13%) and harvest index (0.012%). Above correlations indicate the importance of yield and its relationship with the studied attributes. Since the effective material namely rutin may be regarded as a flavonoid compound and is more likely to be of fundamental application in the molecular structure as sugar, every factor leads to the increase in the openness duration of stomata existing in the plant leaves so that the production of carbohydrates and consequently, the yield and rutin contents (%) will be increased.

Table 2. Mean comparison of grain yield, starch, protein and rutin content of buckwheat.

Treatment	Grain yield (t ha^{-1})	Strach (%)	Protein (%)	Stem rutin (%)	Leaf rutin (%)	Flower rutin (%)
(P)						
P_1	2.021a	47.05a	14.10a	0.10a	0.47 a	0.85a
P2	2.127 a	46.99a	14.00a	0.07a	0.47 a	0.61b
(D)						
D1	1.692 d	46.20d	13.68d	0.05 b	0.48b	0.47d
D2	2.205 c	46.41c	13.79c	0.04b	0.32c	0.99a
D3	2.381 a	46.86b	14.80a	0.06b	0.32c	0.77b
D4	2.363 b	48.62a	13.95b	0.17a	0.65a	0.68c
(N)						
N1	2.070 d	47.17b	13.87c	0.065c	0.42c	0.75ab
N2	2.259a	47.03c	14.08ba	0.088b	0.45bc	0.70cb
N3	2.128 c	4770a	14.07b	0.10a	0.54a	0.79a
N4	2.181 b	46.21d	14.19a	0.088b	0.48b	0.68c
(P×D)						
P1D1	2.097 e	46.79b	13.52e	0.06dc	0.50c	0.46de
P1D2	2.363 c	46.13b	13.61e	0.04dc	0.32d	0.10de
P1D3	2.649 a	46.34b	13.97cb	0.07c	0.47c	1.13a
P1D4	1.655 h	48.85a	14.09b	0.21a	0.60b	0.69c
P2D1	2.073f	46.93b	13.84cd	0.04d	0.46b	0.49d
P2D2	2.398 b	43.27b	14.76b	0.04d	0.33d	0.91b
P2D3	2.313 d	46.48b	14.84a	0.06dc	0.36d	0.41e
P2D4	1.728 g	48.69a	13.80d	0.14b	0.73a	0.66c
(P×N)						
P1N1	1.900 h	48/07b	13.85c	0.07e	0.46bc	0.93a
P1N2	2.314 b	45.68g	13.89c	0.09cd	0.44c	0.89a
P1N3	2.094 f	47.63d	14.08b	0.10b	0.52ab	0.90a
P1N4	2.162 e	46.81d	14.18ba	0.12a	0.47bc	0.64b
P2N1	2.440 c	46.28t	13.86ba	0.05t	0.38d	0.58c
P2N2	2.178 d	48.32a	14.07b	0.48ed	0.45bc	0.52c
P2N3	2.429 a	47.77c	14.31a	0.10b	0.56a	0.68b
P2N4	1.932 g	45.61h	14.19ba	0.04t	0.50abc	0.71b
(D×N)						
D1N1	2.024ac	46.87j	13.23j	0.04ef	0.52ef	0.54gih
D1N2	1.993 ac	47.12h	13.61h	0.06d	0.056de	0.45ij
D1N3	1.663cd	47.62d	13.61h	0.06d	0.60d	0.50ijh
D1N4	2.212ab	45.82l	14.27d	0.03g	0.025ij	0.51jh
D2N1	2.178ab	45.65m	13.89fg	0.05ef	0.32h	0.92bc
D2N2	2.229 ab	47.08l	14.53c	0.04ef	0.34h	0.94bc
D2N3	2.180ab	44.65o	14.79b	0.05ed	0.47ef	1.11a
D2N4	2.320ab	47.44f	14.86b	0.03gf	0.33ih	1.02ab
D3N1	2.240ac	45.04f	14.30d	0.05ed	0.17kj	0.84cd
D3N2	ab 2.332	42.62k	13.40i	0.05ef	0.44gf	0.79ed
D3N3	2.426a	45.09m	15.01a	0.10c	0.71c	0.86cd
D3N4	2.390 ab	44.26q	13.54h	0.05ed	0.36gh	0.59ef
D4N1	1.794 cd	47.56e	13.78g	0.10c	0.86b	0.70ef
D4N2	1.998bc	51.13a	13.94fe	0.18b	0.45gh	0.63gf
D4N3	1.848 cd	48.44c	14.00fe	0.19b	0.37gh	0.68f
D4N4	1.449 cd	47.31g	14.08e	0.23a	0.99a	0.69ef

Means followed by the same letter in each column are not significantly different (p =0.05). P_1 and P_2 were planting treatment mounds with the width of 50 cm associated with two planting rows regarding the distance intervals of 20 cm and those with the width of 60 cm along with three planting rows which are of the distance intervals of 15 cm. D_1, D_2, D_3 and D_4 were sowing date 20th June, 5th July, 20th July and 5th August. N_1, N_2, N_3 and N_4 were nitrogen treatments 0, 50, 100 and 150 kg ha^{-1}.

Starch content (%)

Based on means comparisons, the most starch percent as 48.62% has been attributed to D_4 while with respect to planting pattern of nitrogen, the highest starch content (%) estimated as 48.32, 48.84 and 51.13% were related to P_2N_2, P_1D_4 and D_4N_2, respectively (Table 2). Triple interactions of treatments indicate the highest starch content as 51.69% for the treatment of $P_1D_4N_2$. Environmental conditions have significant impacts on quality and quantity of products during the plant growth period (Inoue et al., 2004). Accumulated starch amounts within the grain depend on the number of endosperm cells which are created in the early stages of pollination and seed filling as well as the rate and time of

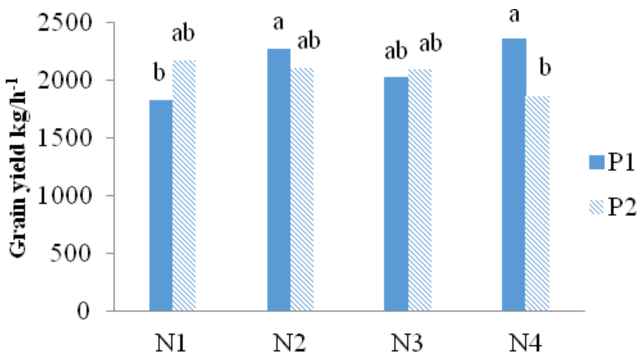

Fig 2. Interaction of planting pattern and nitrogen fertilizer of grain yield.

seed filling period along with the size and composition of mature grains (Cawoy et al., 2008). It seems that although starch content is high because of better environmental conditions (appropriate temperature) in the fourth sowing date as compared to the other ones, starch content decrease as well as the reduction of final starch potential content can be verified because mean temperature is higher in the summer planting during the growth period (Li et al., 2001). In a variety of sowing dates, inappropriate environmental conditions (high temperature) are adjusted so that anatonose phenomenon involving the degradation of larger molecules such as starch and their conversion into smaller ones like glucose and fructose is decreased. Most carbohydrates existing in the grains result from photosynthesis while filling them and retransfer of dry matter into them. Given the growth form of plant (Indeterminate), the stored materials play crucial roles before the beginning of flowering period in order to retransfer of dry matter. Accordingly, it has been found that the shortening of carbohydrates accumulation period should be addressed with respect to the interactions of growth characteristics (Indeterminate) and high temperature in July and August (summer planting). During grain filling period, limitations of carbohydrates accumulation in the grains and protein content will be increased due to the effects of dry and hot conditions on this stage at the end of regarded season while the grain weight is decreased. Effects of sowing date on the other plants' physiologic properties have been reported (Rufang et al., 2010; Hokmalipour et al., 2011). Few researchers have suggested that although the grain weight and yield are reduced because of heat stress, the decrease in grain filling period leads to the decrease in the grain carbohydrates and the increase in protein content unlike carbohydrates (Tahir et al., 2006; Subedi et al., 2007). Effects of environmental elements such as high temperature have been discussed with regard to the lack of endosperm development (Inoue et al., 2004). According to Table 2, there is no meaningful difference between two desired planting patterns showing the direct effects of planting pattern on starch content but the advantages of planting pattern P_1 can be attributed to the indirect effects of planting pattern on starch content of grain and the interaction of sowing date and nitrogen. Nutrients like nitrogen are essential to sustain physiologic processes involving growth controller and development. At the beginning of growth season, nitrogen causes the enhancement of number, size and dry matter value of leaves and petioles and at the end of mentioned season, it also leads to the increase in dry matter value. Meanwhile, nitrate and nitrite restores need a regenerative force resulting from respiration or photosynthesis. If the required force is supplied by the respiration, the increase of nitrogen may reduce carbohydrates. Therefore, using 50 kg nitrogen can provide the highest starch content (Figs. 3 and 4). A negative correlation between protein and starch contents has been discussed (Kalinova, 2005; Ikeda, 1995). Afterwards, Kalinova (2006) observed a positive correlation between protein and starch contents. But it is not in conformity with the result presented by this paper. In this research, a positive correlation was observed between starch content, protein content (-0.095%) and grain yield (0.158%) Table 4. This correlation refers to the significance of carbohydrates content for the grain yield regarding high starch content of buckwheat.

Protein content (%)

As the mean comparisons have reported (Table 2), the highest protein content has been given as 14.80% for the third sowing date. Protein content of 14.19% was achieved using 150 kg nitrogen (N_4). With respect to the planting pattern and sowing date, most protein content estimated as 14.84% was reported for the treatment of P_2D_3. For the planting pattern with nitrogen and sowing date with nitrogen, protein contents of 14.31 and 15.01% have been obtained for the treatments of P_2N_3 and D_3N_3, respectively. Regarding three treatments' interactions, the highest protein content as 15.24% was related to the treatment of $P_2D_3N_4$ (Table 3). Results show that the third sowing date has been accepted as a suitable one because of the appropriate mean temperature in order to store protein. Climatic conditions of summer along with heat stress after flowering stage limit photosynthesis. Under such conditions (reduction of nitrogen absorption after flowering stage due to dryness of soil and inefficiency of roots), it seems that more efficiency of retransfer may be introduced as the fundamental element for increasing the protein content of grain. Genetic features can be addressed as second effective elements in this regard. Also, the shortening of grain filling period, decrease of carbohydrates storage and increase of storage speed of nitrogen materials lead to the enhancement of protein content (%) in the grain. Thus, environmental factors have no significant impacts on the reduction of protein content in this paper. Buckwheat protein content has been estimated as 10.6-15.5%. Another research conducted on buckwheat proposed 13.3% for protein content (Kalinova et al., 2009). The highest protein content of buckwheat has been given as 11.7% (Hore and Rathi, 2002). Furthermore, mean protein content of buckwheat was presented as 10.2-17.9% in relation to the effects of various elements on the grain protein content (Lu et al., 2013). First and second sowing dates result in the decrease of roots development and efficiency of nitrogen absorption as well as

Table 3. Mean comparison among all treatments interaction for grain yield, starch, protein and rutin content of buckwheat.

T reatmen			Grain yield tha^{-1}	Starch content	Protein content	Leaf rutin content	Stem rutin content	Flower rutin content
P_1								
	D_1	N_1	2.19 bh	49.19 af	13.26 jk	0.69 ih	0.05 lk	0.56 ef
		N_2	2.15 bh	42.97 kl	13.42 hk	0.50 lm	0.09 ef	0.55ef
		N_3	1.71fh	47.42 bj	13.41 hk	0.42 po	0.08 ef	0.59d
		N_4	1.793 f	45.25 gl	13.94 fh	0.42 po	0.03 on	0.30mk
Mean			1.94	46.20	13.50	0.50	0.06	0.50
	D_2	N_1	1.65 fj	44.03 il	15.14 ab	0.99 c	0.07 ij	0.21mk
		N_2	1.07g	45.18 el	14.58 ce	0.99 c	0.04 ln	0.33hk
		N_3	2.27 bf	43.62 kl	14.95 ac	1.16 b	0.04 ln	0.35 hi
		N_4	2.00bf	50.14 ad	14.74 ad	1.09 b	0.02 o	0.32 jk
Mean			1.74	45.74	14.85	0.99	0.04	0.30
	D_3	N_1	1.70ej	47.63 ai	14.96 ac	0.35 pq	0.05 l j	0.15 ml
		N_2	2.33 af	47.96 ai	13.28 jk	1.29 a	0.05 lk	0.43 hf
		N_3	1.99 j	50.75 ac	14.41 dg	1.36 a	0.32 a	0.83 bc
		N_4	2.16 ch	44.16 il	13.54 hj	1.30 a	0.08 ih	0.42 hf
Mean			2.04	47.62	14.04	1.07	0.12	0.45
	D_4	N_1	1.71 ej	46.88 cj	13.92 fj	0.89 d	0.11 d	0.92 ba
		N_2	1.71 ej	51.69 a	14.08 eh	0.73 gh	0.17 c	0.49 ef
		N_3	1.60 hj	49.05 af	13.86 fj	0.66 ij	0.19 b	0.18 mk
		N_4	1.35 hj	47.99 ag	14.62 ae	0.36 pq	0.10 ef	0.93 ba
Mean			1.59	48.90	14.12	0.66	0.14	0.63
P2								
	D_1	N_1	1.80 bi	44.69 hl	13.23 k	0.58 l j	0.04 ln	0.48 hf
		N_2	1.31 hj	51.41 ab	13.74 hj	0.46 no	0.04 ln	0.52ef
		N_3	2.06 bi	47.99 ag	13.83 hk	0.55 lm	0.05 lk	0.54 ef
		N_4	2.03 bi	44.69 hi	14.61 ae	0.36 pq	0.03 on	0.24 mk
Mean			1.59	47.19	13.85	0.48	0.04	0.44
	D_2	N_1	2.06 bi	46.54 cj	13.57 gk	0.83 df	0.02 o	0.22 mk
		N_2	1.73 fj	47.41 cj	14.48 ce	0.86 de	0.05 lk	0.31 jk
		N_3	2.20 ae	49.04 ah	14.67 ae	0.98 c	0.06 ij	0.50 ef
		N_4	2.19 ae	45.85 gl	15.03 ac	0.92 dc	0.04 ln	0.31 jk
Mean			2.04	47.21	14.43	0.89	0.04	0.33
	D_3	N_1	1.48hj	42.57 l	13.67 gk	0.30 q	0.05 j	0.15 m
		N_2	2.20 ah	44.69 hl	13.56 gk	0.27 q	0.05 lk	0.41 hf
		N_3	2.45a	49.58 ae	13.67 gk	0.52 lm	0.10 ed	056 ef
		N_4	1.99 di	44.51 hl	15.24 a	0.61 i j	0.30 mn	0.29 mk
Mean			2.03	50.72 a-d	14.03	0.42	0.12	0.35
	D_4	N_1	1.31jh	48.43 af	13.67 gk	0.52 lm	0.93 ef	0.52 lm
		N_2	1.57 bi	47.97 ai	13.82 gk	0.56 lm	0.19 b	0.56 lm
		N_3	1.67 fj	42.57 l	14.16 dg	0.74 gf	0.19 b	0.74 gf
		N_4	1.54gh	44.69 hl	13.6 gj	0.79 gf	0.08 gf	0.52 lm
Mean			1.52	45.91	13.81	0.62	0.34	0.75

Means followed by the same letter in each column are not significantly different (p =0.05). P_1 and P_2 were planting treatment mounds with the width of 50 cm associated with two planting rows regarding the distance intervals of 20 cm and those with the width of 60 cm along with three planting rows which are of the distance intervals of 15 cm. D_1, D_2, D_3 and D_4 were sowing date 20th June, 5th July, 20th July and 5th August. N_1, N_2, N_3 and N_4 were nitrogen treatments 0, 50, 100 and 150 kg ha^{-1}.

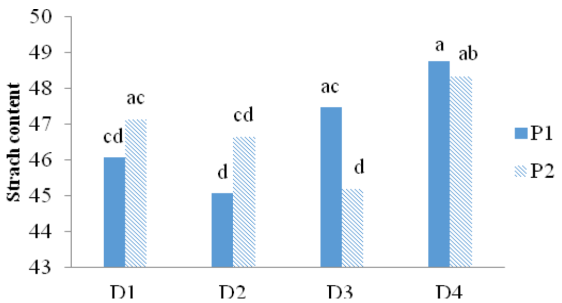

Fig 3. Interaction of planting pattern and sowing date of starch content.

the increase of heat stress effects on grain filling period and the changes in the optimal conditions of plant growth may finally reduce the grain protein content. Fourth sowing date increases the carbohydrates storage and decreases the storage speed of nitrogen materials due to suitable environmental conditions and the lengthening of grain filling period; in other words, they result in the increase of grain starch content and the decrease of protein content. Increase of protein accumulation in the grain and decrease of carbohydrates accumulation speed are more likely to be discussed as the most important factors to increase protein content under heat stress at the end of desired season (Panozzo and Eagles, 1999). Such conditions as high to moderate densities or appropriate distribution with constant density can enhance the grain protein content (Zakarakas, 1999; Zhang et al., 1998; Zhang et al., 2002). This event has occurred in the planting pattern of P_2. Reasons of an increase in grain protein content in this planting pattern (uniform) in comparison with the other ones are addressed as the decrease of canopy cover and not-impaired nitrogen storage and amino acid cycles caused by the increase in nitrate rate. In addition, it should be noted that the competition of assimilates, particularly nitrogen is decreased in this planting pattern and as a result, more levels of nitrogen are allocated to grains leading to the enhancement of grain protein content. According to the given results, most effects of protein content increase are imposed by the use of 150 kg nitrogen fertilizer that is in accordance with the other records (Doyle and Holford, 1993; Subedi et al., 2007). It has been understood that the increase in the redistribution of nitrogen in fertilizer treatment using 150 kg ha^{-1} nitrogen and its interaction with the shortening of grain filling period because of heat stress impacts at the end of regarded season can significantly enhance the efficiency of mentioned fertilizer treatment as compared to the other ones. Reduction of agricultural efficiency of nitrogen usage due to the increased consumption of nitrogen fertilizer can be related to the increased speed of desired element losses through leaching, sublimation and failure to absorb effectively. Therefore, it can be stated that although the increase of nitrogen percent is followed by the increase of protein content, the enhancement of agricultural efficiency of nitrogen consumption is considerably important while having a positive correlation with grain yield. Considering high protein content (5) of this plant with respect to genetic aspects, the interactions of temperature and effective nitrogen absorption along with grain protein content have been specified as the factor altering the experimental nitrogen treatments. In this regard, researches have reported the effects of lots of elements on protein content. The most important one is recognized as nitrogen fertilizer amount (Feng et al., 2003; Chai et al., 1998; Zhang et al., 2001). The research findings on the impacts of nitrogen amounts on grain protein content in the most plants such as the studied one are confirmed by the results of other studies. Grain protein content is considerably correlated with the usable nitrogen of soil and the necessity of nitrogen fertilizer consumption on the basis of plant needs has been verified (Feng et al., 2003; Chai et al., 1998; Zhang et al., 2001). Several reports on the effects of many factors on the increase of protein content have specified the impacts of density and nitrogen fertilizer (Feng et al., 2003; Chai et al., 1998; Zhang et al., 1997, 1998 and 2001). In some researches, it has been discussed that the correlation of grain yield and grain protein content is meaningful but negative. Subedi et al., (2007) have investigated the environmental conditions' effects on grain filling period in the sowing date of Mid-May on the rows having the distance of 12.5 cm with density of 200 bushes per hectare and furthermore, they have observed the correlation of protein and starch contents (Kalinova et al., 2006). Kalinova et al. (2005) and Ikeda (1995) reported the negative correlation of protein and starch contents (Kalinova et al., 2005; Ikeda, 1995). In this experiment, the correlation of protein content and grain yield is not meaningful (0.111%).

Leaf rutin content (%)

According to table 3, the highest leaf rutin content as 0.99 m gr of dry matter is attributed to the treatment of $P_2D_4N_4$. Results indicate that suitable temperature and day length increase the length of growth period and leaf area index through enhancing the number and area of it in the fourth sowing date. In first and second sowing dates, high temperature causes the decrease of leaf rutin content (%) as compared to the vegetative stages. Thus, leaf rutin content experiences its highest percent during the above-mentioned period in comparison with the other ones. Rutin extraction and measurement in the plants obtained from different cultivations showed that the current rutin content of the leaves will be decreased as the weather gets warm and the day length increases so that the highest and lowest contents can be determined for the planting periods of March and July (Omidbeigi and Zakizadeh, 2002). Examining the buckwheat rutin content in different sowing dates, it has been reported that rutin content in the sowing dates after summer is the half of that in the summer ones. They proposed a positive correlation between the days of sowing date and rutin content. Studies show the rutin production increase at higher temperatures (Mao et al., 2003). Studies done by Handa and Kaul have indicated that the rutin production may be increased when the temperature is high. But very high temperature causes the burning of leaves and fagopyrum vegetative organ so that the yield and quality of vegetative organs as well as rutin content (%) are reduced (Handa and Kaul, 1996). Examining 4 cultivars of fagopyrum has been done under the controlled conditions and a short photo-period (12 hours) or a long one (19 hours) during day and night at 24.5 and 18°C or 18 and 12°C, respectively. Results showed that the maximum yield of dry matter has been obtained from hot and long days. Flowers and leaves' rutin contents followed the same trend and the highest rutin content estimated as 5% was achieved from one of the cultivars during hot and long days (Seong et al., 1998). It has been reported that among the environmental factors, the most effective one in the accumulation of flavonoids and rutin is temperature (Omidbeigi, 2005). In addition to temperature, light is also of high importance (Lee et al., 2001). Establishing a general relationship between fine and gross production rates in this plant can lead us to gain the best accumulation and planting pattern with respect to the total leaf index specified as a criterion to measure photosynthesis and biomass production (including metabolic biomass i.e. optimum rutin production). In this respect, the planting pattern of P_2 (60 cm mounds with 3 rows and 15 cm distance intervals) may enhance the eco-physiological relationships between the inputs and consequently, lead to the same results regarding the metabolic biomass production (rutin). Oat et al. (1999) have estimated the mean flavonoid and rutin contents in the buckwheat as 1314 to 387 m gr and 47 to 77 m gr for 100 gr dry matter. Omidbeigi and Zakizadeh (2002) studied the effects of different planting accumulations on vegetative and generative yield and rutin contents in the buckwheat plant and found that the density of

Table 4. Correlation coefficients between grain yield, starch, protein, leaf rutin, stem rutin, flower rutin content of buckwheat.

	Grain yield	Starch content	Protein content	Leaf rutin	Stem rutin	Flower rutin
Grain yield	1					
Starch content	0.158**					
Protein content	0.111 n.s	-0.095**				
Leaf rutin	0.396 n.s	-0.102 n.s	0.07 n.s			
Stem rutin	0.369 n.s	-0.112 n.s	-0.017**	-0.465**		
Flower rutin	0.163*	-0.062 n.s	0.130 n.s	-0.046 n.s	-0.122 n.s	1

* p<0.05, **p<0.01 and , ns= non-significan

33.3 bushes per m^2 had the highest amount of dry matter in the vegetative body while having the lowest rutin content (%). However, the accumulation of 100 bushes per m^2 showed the reversed results. These results represent the reversed relationship between dry weight and vegetative yield with the rutin content (Figs. 3 and 4). Regarding the results of nitrogen fertilizer levels and leaf rutin content, it can be seen that 150 kg nitrogen can increase the vegetative sprouts on the branches and leaf production in the plant; in other words, the leaf rutin content (%) will be enhanced as compared to nitrogen treatments of 0, 50 and 100 kg. In an experiment, the effects of nitrogen and protein content in the buckwheat were trivial and non-meaningful (Michalova, 1997). Honermier and Wagenbreth. (2000) reported the increase of rutin content as 18 to 29% in the buckwheat using 0 to 90 kg ha^{-1} nitrogen. Studying 7 cultivars of buckwheat and 2 levels of nitrogen fertilizer, the highest leaf rutin content was measured as 0.61% for the cultivar of aelita using 50 kg nitrogen (Kalinova et al., 2006).

Correlation coefficients of plant properties in table 4 have been demonstrated that the leaf rutin content has a positive correlation with the rutin contents of grain (0.06%), flowers (0.13%) and harvest index (0.012%) while negatively correlated with the stem rutin content (-0.09%). Above correlations are the indicatives of plant rutin content and its relationships with all the studied properties. Given that the grain is the product of photosynthetic activities of vegetative organs especially leaves, high correlations of these qualities should be expected indicating that the plants having good chlorophyll development and appropriate vigor are required in order to achieve higher yields followed by more numbers of flowers and grains and higher harvest index. Negative correlation of stem rutin content suggests that more leaves (leaf to stem ratio), more stored dry matter in the leaves resulting in the enhancement of leaf rutin content as compared to the stem. Besides, most of red light may be absorbed by the upper parts of each bush and the light absorbed by the lower ones considerably contain the wavelengths of blue and ultraviolet spectra. It is likely to increase the rutin content of lamina. This result is confirmed by the findings on higher leaf rutin content as compared to the stem reported by Li et al. (2014).

Stem rutin content (%)

As table 3 has already shown, the highest stem rutin content was given as 0.37 m gr for 100 g dry matter relevant to the treatment of $P_1D_4N_4$ with regard to the triple interactions of treatments. This result suggests that the fourth sowing date was more suitable because of temperature balance between the vegetative activities and necessary enzymes (secondary metabolism). Regarding the other sowing dates, high temperature and the increase of respiration as well as the decrease of photosynthesis have caused the reduction of stem diagonal and height along with the storage capacity. An experiment conducted on buckwheat rutin content concerning different sowing dates has presented that rutin content for the sowing dates after summer is half of that for summer cultivation. Also, a positive correlation was observed between the days of sowing dates and rutin content (%) (Li et al., 2008). The best planting pattern has been introduced as the one which can be associated with the most appropriate bush distribution in order to achieve high rate of production and the optimum use of existing inputs. In this regard, the planting pattern of P_1 (50 cm mounds with two rows and intervals of 20 cm) has been able to keep balance between the intakes and the formation of maximum leaf area index as a production unit which has consequently related to high number of stems, thicker ones and finally, high storage rate of rutin content. Studying 7 cultivars of buckwheat with 2 levels of nitrogen fertilizer (0 and 50 kg) to measure the rutin contents of stem, grain, leaf and flower on the basis of 100 g dry matter, the highest content as 0.006% was related to the stem of krupinka using 50 kg nitrogen (Kalinova et al., 2004). Kreft et al. (1999) reported the effects of nutritional ingredients on the rutin contents of grain, leaf, flower and stem in the buckwheat as the mean rutin contents of leaf, stem and flower were 300-46000 ppm. The effects of nutrition on buckwheat rutin content have been investigated and measured in a variety of cultivars (Kim et al., 2004; Williamson et al., 2000; Kitaboyashi et al., 1995 and Michalova et al., 1998). Schnieder et al. (1996) have studied the effects of temperature degree on the rutin content and obtained the highest rutin contents at the maximum temperature of 24.5 and 18 ºC and the minimum temperature of 18 and 12 ºC for the day and night. It was stated that the relationship of nitrogen fertilizer and rutin content in the buckwheat was not significant (Marquard and Kroth, 2001). Hore and Rathi (2002) reported the 18 to 29% increase of rutin content in the buckwheat using 0 to 90 kg ha^{-1} nitrogen. While investigating specific cultivars and nitrogen fertilizer levels of 0-50 kg, it has been demonstrated that the interactions of flower, stem and leaf rutin contents were meaningless using nitrogen fertilizer (Kalinova et al., 2004). Other researchers believe that the rutin extraction and the growth and environmental conditions of the studied area are likely to affect the results of rutin content (Kavacevic and Rode, 1998; Kreft et al., 1999). Kwang Jin et al. (2004) have conducted a study on 2 cultivars of buckwheat with 4 densities and 5 levels of fertilizer and concluded that the highest rutin content as 1887 mg for 100 gr dry matter could be relevant to the treatment of 30×60 and the highest rutin contents in 2 cultivars were 1839 and 1934 mg without using the fertilizer. Table 4 indicated four correlation coefficients of plant properties and presented that stem rutin content has a positive but meaningless correlation with the grain yield (0.28%). Above correlation shows that the increase in the yield indirectly influences the increase of stem rutin content. More and thicker pedicles and lateral stems resulting from the higher levels of stem growth due to the availability of nutrients for generating more boughs and leaves (the higher

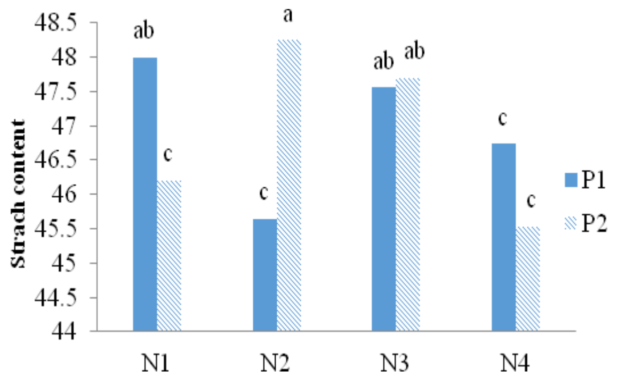

Fig 4. Interaction of planting pattern and nitrogen fertilizer of starch content.

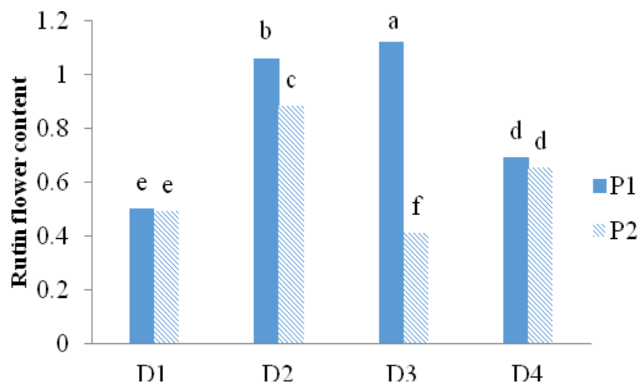

Fig 5. Interaction of planting pattern and sowing date of flower rutin content.

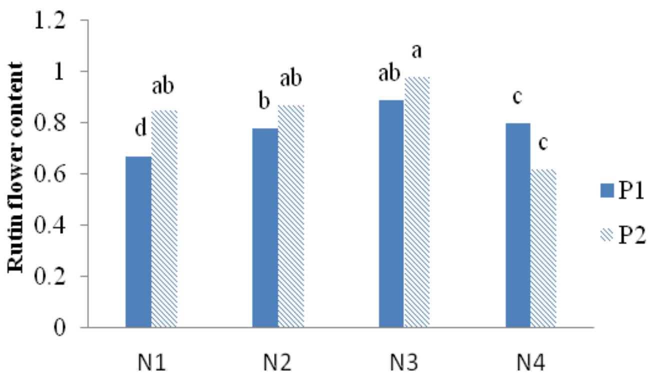

Fig 6. Interaction of planting pattern and nitrogen fertilizer of flower rutin content.

storage contents of rutin) and as a result, the enhancement of photosynthesis and the remobilization of dry matter into the grain can play effective roles in increasing the yield.

Flower rutin content (%)

As table 3 has already shown, the highest flower rutin content was given as 1.37 m gr at 100 g dry matter relevant to the treatment of $P_2D_3N_3$ regarding the triple interactions of treatments. Considering the accumulation of secondary metabolites, it can be concluded that when first metabolism having the maximum activity rate finishes the plant vegetative growth, a great deal of resultant materials and energy will be transferred to activate the generative and flowering stages. If the plant encounters the environmental stresses including a temperature one, it will be forced to consume the physiological storages during a vital stage and instead, high amounts of remaining obtained from first metabolism may be remained as secondary metabolites. These metabolites will considerably concentrate and accumulate in some specific points of plant vegetative body. As mentioned before, this phenomenon is of lots of economic consequences (Morishita et al., 1995). It has been confirmed in the third sowing date with the planting pattern of P_1 (50 cm mounds with 2 planting rows and the intervals of 20 cm) using 100 kg nitrogen. During a 2-year experiment, the changes of sowing date and planting pattern and those of sowing date and nitrogen fertilizer showed that the interactions of year with planting pattern and year with sowing date are meaningful. Therefore, planting pattern P_1 has the most appropriate conditions for the third sowing date and suitable use of 100kg nitrogen to obtain the highest rutin content of the plant among the interactions of treatments (Figs. 5 and 6).

Regarding first and second sowing dates, the number of generative units and flowers and flower rutin content are reduced due to high temperature during vegetative and generative stages and lack of respiration balance between the production and consumption. Investigating the effects of temperature degree on the rutin content (%), the highest content was given for the maximum temperature degrees of 24.5 and 18 ºC and the minimum one for 18 and 12 ºC during the day and night, respectively (Seong et al., 1998). In the planting pattern of P_1 (50 cm mounds with 2 planting rows and the intervals of 20 cm), generative stage will begin earlier due to high contest of single-bush plants and the maximum number of flowers is achieved while harvesting the flowers to measure the rutin content. Omidbeigi et al. (2004) performed an examination on the buckwheat yield of 7 cultivars in various sowing dates in Iran and stated that 25th June is the best sowing date with respect to the rutin content. Also, the highest rutin content as 1.21% has been reported (Marquard and Kroth, 2001). Williamson et al. (2000) examined a variety of buckwheat cultivars and measured the highest flower rutin content as 12 m gr. With respect to the effects of nitrogen on the flower rutin content in the buckwheat, it has been found that high levels of nitrogen reduce the flower rutin content and its changes will follow the mitscherlich falling efficiency to a given extent as the nitrogen consumption is enhanced. In this regard, the plant has been able to give the highest flower rutin content using 100 g nitrogen as compared to the other treatments. In a study, it has been stated that there is a meaningless relationship between nitrogen and rutin content of buckwheat (Mikalova, 1997). Honermeier and Wagenbreth (2000) have proposed the 18 to 29% increase of buckwheat rutin content using 0 to 90 kg nitrogen. Studying 7 cultivars of buckwheat with 2 levels of nitrogen fertilizer (50 kg) for the measurement of rutin contents of grain, stem, leaf and flower on the basis of 100 g dry matter, it was obtained that the highest flower rutin content as 65% was relevant to Kora (Kalinova et al., 2004). They also stated that using nitrogen fertilizer had a meaningless relationship with the flower, stem and leaf rutin rates while having a significant relationship with the grain rutin content. Kreft et al. (1999) studied the effects of nutritional ingredients on the rutin contents of grain, leaf, flower and stem in the buckwheat and reported the average rutin contents of leaf, shoot and flower between 300-46000 ppm. Rutin content of various buckwheat parts was measured and the highest content was recorded for the flower (Cawoy et al., 2009). Furthermore, it has been suggested that polyphenolic compounds in the medicinal plants decreased before and after the flowering stage (Grevsen et al., 2009). It is in conformity with the result found in this research. Table 4 has shown four correlation coefficients and proposed that flower rutin content is significantly correlated with the grain yield (0.138%). It can be concluded that the increase in the number of ears will lead to produce more flowers in the plant and as a result, flower rutin content and the yield are enhanced.

Materials and Methods

Case study

Field experiment was conducted by Agricultural Research Institute of Arak, Iran in a specific area located in northern latitudinal $34^{o}\ 5^{'}$ and eastern longitudinal $49^{o}\ 42'$ that is 1757 m above sea level during 2010-2011. The soil is of a clay-loam texture, pH of 7.6, the electrical conductivity of water saturation computed as 1.28 DS m^{-1} and soil nitrogen content of 0.06% (Table 1). According to weather data, mean annual temperature and precipitation were 24.6°C and 0.00 mm, respectively (Soil Survey Staff, 2006).

Methodology

The experiment was conducted as the completely randomized blocks in the form of split plot factorial with three replications. Planting treatments (P) as the fundamental element were implemented at two levels including the mounds with the width of 50 cm associated with two planting rows regarding the distance intervals of 20 cm (P_1) and those with the width of 60 cm along with three planting rows which are of the distance intervals of 15 cm (P_2). Sowing dates and nitrogen treatments as the minor elements were examined for four dates and weights involving 20th June (D_1), 5th July (D_2), 20th July (D_3) and 5th August (D_4) and 0 kg ha^{-1} (N_1), 50 kg ha^{-1} (N_2), 100 kg ha^{-1} (N_3) and 150 kg ha^{-1} (N_4), respectively. With respect to the fixed density of 100 bushes per square meter, the distances between the planting lines were specified as 4 and 5 cm for the treatments of P_1 and P_2, respectively. Dimensions of each plot for the planting patterns of P_1 and P_2 have been determined as 1.6×2 and1.6×2.4 m consisting of four planting rows. Planting operations were done by hand regarding the above-mentioned sowing dates. Distribution of nitrogen fertilizer (on the basis of urea fertilizer) was done in 2 stages: first and second stages included 4-leaved and 8-leaved plants, respectively.

Measurement of Studied Properties

To calculate the final grain yield, the sum of existing seeds per a square meter (based on 12% humidity) regarded as the grain yield criterion was computed when maturity of 75% seeds was achieved in every plot. Rutin extraction and measurement has been performed by the means of HPLC device (Kreft et al., 1999-2002; Dadakova and Kalinova, 2010). After injecting and drawing the desired graphs, the exact rutin content (%) of plant samples was estimated by the use of obtained values and the following equation (Omidbeig and Mastro, 2004):

$$\text{Rutin}(\%) = \frac{a \times V}{v \times m} \times 100 \qquad (1)$$

Where, a: rutin content (mgr.) reported by HPLC device
V: final mass (mL) obtained from the extraction
v: injection mass (mL) of the desired sample
m: dry weight (mgr.) for the extraction
In order to determine starch contentof the harvested seed samples in each plot, 100 g seeds were sent to Agriculture Organization of Arak and each sample's starch content (%) was separately measured using NMR device in the laboratory. Furthermore, Kjaldahl method has been utilized to measure the content of macro elements and afterwards, grain protein content has been estimated through the following equation:
Grain protein content (%) = grain nitrogen content (%) × 6.25 (Lu et al., 2013)

Statistics Analysis

All statistics and variance analyses have been conducted by the help of SAS software (Version 8.2) and a graph has been drawn by Excel software. Resultant means were examined by the means of multiple-range Duncan test at the possibility level of 5% (SAS, 2002).

Conclusions

Research results indicate that third and fourth sowing dates are more appropriate for the regions with hot summer like Arak in Iran regarding qualitative and quantitative yield and accumulation of rutin from first and second sowing dates. Also, nitrogen usage amount (100 kg h^{-1}) with the planting pattern P_2 (mounds with the width of 60 cm and 3 rows) is suggested.

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