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Evaluation Anatomy of Flag Leaf of Rice Cultivars Affected Different Planting Dates under Dry and Warm Climate condition in Southwest of Iran

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ABSTRACT

Current research was conducted during 2006 and 2007 cropping seasons to assessment the anatomy of the flag leaf and yield of rice in the Khuzestan province according split plot experiment based on randomized complete block design with three replications. Main plot included different sowing dates at three levels (5 May, 26 May and 16 June). Hoveizeh, Hamar (Heat tolerance), Ghermez Anbori, Champa (Heat sensitive) and Danial (Relatively heat tolerant) cultivars at five levels were belonged to sub-plots. The results of combined analyses showed that was a significant different in planting date by variety interaction in all of trades. On the other hand, the related temperature condition, varieties had different reaction. Given that metabolic energy is needed to transfer sugar in this path, reduction of phloem cell surface can predispose the material preservation and shortening the transfer path by reducing the rate of respiration and transfer intervals. In the other hand, size of phloem depends on the size of source and reservoir and their relationships. Highest and lowest levels of large vascular bundles were related to 2nd and 1st planting dates; but highest and lowest levels of small vascular bundles, xylem and phloem obtained in 2nd and 1st planting dates respectively.

Keywords: *Genotypes, Morphology, Oryza sativa, Sowing date.*

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INTRODUCTION

Rice is one of the world's most important staple food crops. In Asia, it is the main item of the diet of 3.5 billion people. Therefore, the increase in population will require 70 percent more rice in 2025 than is consumed today (Lack *et al*., 2012; Moosavi *et al*., 2015). The principal objective in the modern agricultures is better the understanding of crop's growth and increment for the optimal usage of the natural resources and consequently reaching greater yield. Accordingly, suitable planting date is one of the major factors in efficient agricultural management playing a significant role in the production control (Dinesh *et al*., 1997). The sowing date of the rice crop is important for three major reasons. Firstly, it ensures that vegetative growth occurs during a period of satisfactory temperature and high levels of solar radiation. Secondly, the optimum sowing date for each cultivar ensures the cold sensitive stage occurs when the minimum night temperatures are historically the warmest (Farrell *et al*., 2003). Ideal sowing date for one or more variety allows for availability of a set of environmental factors that favor a desirable greening, establishment and survival of the plantlet which as a result the plant encounters favorable environmental conditions and the avoid unfavorable ones during each stage of its growth (MacKinnon and Fettel, 2003). Planting date in comparison with other agronomic factors has more influence on the phenologic and physiologic characteristics of the plant, determining the proper planting date makes the maximum coordination between plant growth process and climate conditions (Khayat *et al*., 2014). Alizadeh and Isv (2006) stated that the growth season duration and temperature average have significant effects on rice yield during the different growth stages; therefore, planting date plays a substantial role in rice crop production. Dehghan (2007) mentioned that the variation in planting date might influence crop yield through affecting phenology in the vegetative and the reproductive growth stages. Butler *et al*. (2002) reported that climatic factors are among uncontrolled agents whose individual variations lead to changes in crop growth conditions. Therefore, it is necessary to determine the thermal requirement of the different phenology stages in order to maximally benefit from crops' production potential. The select the appropriate planting date for the planting of rice varieties are genetic factors, the optimum use of capacity. Suitable for the planting on the optimum efficiency of the factors affecting the performance (Ali and Rahman, 1992). Forming properties of the building plants on the ecological behavior, environmental requirements and compatibility with the surrounding the plants have the significant effect. Used of features construction plant morphology is nearly a century ago (Metcalf and Chalk, 1950). About forty years ago, great the changes occurred in the study of structure forming the plants and the plant classification was used in this science. Some scientists have stated that the structural features of the plants are important in the response of plants with different conditions. Today all aspects of building forming plants considered by plant morphology and the results obtained in this area is very important constituent of the traits associated with building plants to the achieve optimum performance with respect to the different environmental conditions that gives (Rudall, 1994; Heywood, 1985; Cutter, 1971; Carlquist, 1961; Ali *et al.,* 1989). The effect of the environmental conditions on the stomatal frequency. Leaves of

plants in arid environments and can grow in high light has smaller holes and more than shade leaves of plants that are grown in the humid environments. The number of stomata in the leaves of a plant will not only single but also is different in different parts of a leaf (Rudall, 1994; Heywood, 1985). Although limited information from the constituent building plants because there is very little research the general symptoms of increased heat resulting changes in the level of a plant include: reduced cell size, stomatal closure and reduce the water losses, increased stomatal density and fuzz, a wooden vessel is larger than the roots and aerial parts (Anon *et al.,* 2004). Several studies revealed that the heat tolerant crop plants have the higher stomatal density, stomatal aperture less leaves thicker, the more compact arrangement of the mesophilic cells, vascular bundles are the more fully developed and the stable structures are cellular organelles (Han *et al.,* 1997). Results of an investigation related to late planting of rice agriculture in China showed that the effect of the high temperature air, chemical and biological processes within the chloroplasts of leaf cells of rice were damaged and there were some differences between the rice varieties. Microscopic study of flag leaf structure of Line No. 996 (Tolerant) and 4628 are sensitive to heat thermal stress (37.30° C) can be found online in China suffered a well-developed vascular bundles in the middle vein, also has a large vascular bundles (With 2.0105 square micrometers) and mesophilic cells with a very strong bond they had together. The openness of the openings in the thermal stress tolerance in very low numbers and even some of them were completely closed, and while this was not developed in the vascular bundles were not only sensitive, but the openings were usually open space the cell was very high in mesophilic cells (Zheng *et al.,* 2009). Crop response to low temperatures depends on weather conditions from which they originated, there. Tropical and subtropical plant origin such as rice, are very sensitive to low temperatures and its growth in the face with a temperature below 15 degrees Celsius are difficult (Allen and Ort, 2001). The response of plants sensitive to frost, is the rapid increase in photosynthetic inhibition (Ort, 2002). Bell and colleagues (Zheng *et al.,* 1998) reported that the structure of rice flag leaf to flag leaf temperature, the amount of damage to the chloroplast structure is different. Limouchi *et al.* (2013) with reviews on the flag leaf vascular bundles, while the announced tolerant cultivars of rice has the potential to increase drug levels are smaller vascular bundles, found that the rice planting in the warmer months of the year to maintain the surface potential of the vascular bundles water, increasing the efficiency of the photosynthesis and respiration rates, is less. He continued failure to affect the number of vascular bundles and cell buliform influence of environmental conditions (Depending on genotype) was proven. Limouchi *et al.* (2013) also the examined different planting dates on ten varieties of rice, reduce the period to reduce the transmission of the data to nonstructural carbohydrates to the main reservoir of seed yield loss as the factors. Grain structure of the constituent elements of the flag leaf is important in influencing production could eventually play an important role in the provision of dry matter for grain. But this effect can be quite affected by the environmental conditions. Details of the constructive elements and plant tissues, a better understanding of the certain actions and adapt to the different environmental

conditions, provides. With out the knowledge of the structure (Anatomy), plant physiological processes within plants are not understood and cannot be designed experiments in physiology and ecology. This research was carried out to evaluate effect of several sowing time on building the flag leaf affected different rice cultivars under different conditions.

MATERIALS AND METHODS

Field and Treatments Information

This study reviews the anatomy of the flag leaf and yield of rice in the Khuzestan province with longitude 48˚, 28″ and latitude 31˚, 50″ with 33 m above sea level, clay soil loam pH near 7.2 and electrical conductivity of 3.1 $ds.m^{-1}$. Current research was conducted during 2006 and 2007 cropping seasons according split plot experiment based on randomized complete block design with three replications in plots of size 2.5×4m. Main plot included sowing dates at three levels (5 May, 26 May and 16 June). Hoveizeh, Hamar (Heat tolerance), Ghermez Anbori, Champa (Heat sensitive) and Danial (Relatively heat tolerant) cultivars at five levels were belonged to sub-plots. Average monthly temperature mentioned in the table 1.

Table 1. Average of min and max monthly temperature degree (planting to harvesting) during crop years of 2006 and 2007 at Shavoor agriculture researches station

Month	2006		2007	
	Mean Min. (ºC)	Mean Min. (ºC)	Mean Min. (ºC)	Mean Min. (ºC)
May	20.7	39.2	21.3	39.9
Jun.	24.8	46.3	24.8	44
Jul.	27.6	47.2	26.5	48.3
Aug.	30.9	45	25.5	47.2
Sep.	20.9	43.1	23.5	43.2
Oct.	20.2	36.4	15.2	36.5
Nov.	-	-	10.5	32.3
Average	24.2	42.9	21	41.6

Farm Management

Rate of consumption of 80 kg seed hac^{-1} and the sprinkling of seeds germinated in soil saturated with water and the combination of hand weeding and weed control using 2-4-D, the value 1.5 lit ha^{-1} tooked control. The nutrient requirements based on the result of soil properties (Table 2) were determined and used, the source of phosphorus, ammonium phosphate and the 50 kg ha^{-1}, 100 kg ha^{-1} of potash from potash and zinc sulphate 40 kg ha^{-1} basis and when sprayed before planting the seeds were consumed. Source of urea nitrogen per 180 kg ha^{-1} of which 50% in three to four leaf stage and 50% remaining at the end of tillering and the beginning of pregnancy were used.

Table 2. Soil characteristics of the research farm

Soil texture	Absorbent micro Elements (ppm)				K (ppm)	P (ppm)	N (ppm)	EC	pH	Soil depth (cm)
	Fe	Mn	Zn	Cu						
Clay-loam	15.7	3.2	2.8	2.8	360	10	0.08	3.1	7.2	0-30

Measured Traits

For measurement of the anatomical features, were isolated at the time of ear emergence and flag leaf of the plant after removal of the terminal and basal segments, cut into 2-3 cm length were taken from the middle part of flag leaf. For maintenance of laboratory samples and send them to the F.A.A's solution was used. In laboratory, and a narrow cross section 10×10 mm using a manual method polystyrene were prepared and stained, samples after washing with distilled water for 15 minutes in bleach water and then 20 minutes in Carmen aluminize and finally for ten to fifteen seconds at each of stages were methyl green staining were washed thoroughly with distilled water. Then samples for preparation of stained slides and pictures were placed on slides and attributes include: the level of large and small vascular bundles, xylem and phloem, wood, large and small vascular bundles and cell number per unit area using a light microscope buliform via 10-40 zoom were measured.

Statistical Analysis

The data were analyzed via MSTAT-C and Minitab software and mean comparison was done via Duncan test at 5% probability level.

RESULT AND DISCUSSION

Large vascular bundles surface: flag leaf is one of main photosynthetic organs in wheat and, because of their location at the top node of stem, is more exposed to environmental stresses such as heat. Change of its anatomic structure is the important approach related to the heat stress. In the present study, it is indicated that effects of planting date, cultivar, interaction effects between the cultivar and year are significant at one percent level and interaction effect between cultivation and year and simultaneous effect of the 3 factors are significant at 5% level (Table 3). Given the comparison of averages, highest and lowest large vascular bundles surface are related to the second and third dates. Among cultivars, Danial had the lowest surface (Table 4). Interaction effect of two factors indicated that all cultivars have the lowest surface at the first cultivation date and the highest surface is belonged to Hamar and Anbori cultivars at the second cultivation date (Table 5).

Table 3. Combined ANOVA of anatomy characteristics flag leaf in experiment treatment

S.O.V	df	Large vascular bundles surface	Small vascular bundles surface	Xylem surface	Phloem surface
Year (Y)	1	7680963.596	872209.077**	1399694.060**	74170.569
Error I	4	2329788.290	26906.996	19221.729	24049.661
Sowing date (SD)	2	30682228.819**	419211.332	122225.827	885753.517**
SD × Year	2	6809653.732*	79817.907	272819.703*	444335.087**
Error II	8	895782.340	151416.368	57567.770	29513.346
Cultivar (C)	4	11859142.597**	115742.606**	601757.672**	886694.755**
Cultivar × Year	4	8801006.692**	1065830.648**	421683.674**	700527.849**
SD × C	8	5222536.310**	667648.738**	118182.194*	896904.282**
SD × Y × C	8	1898282.981*	443274.458**	158078.481**	643456.115**
Error III	48	884806.029	72364.383	42268.486	54694.458
C.V. (%)	-	9.85	17.57	21.76	21.60

^{ns}, * and **: Non significant and significant at 5 and 1% level of probability, respectively.

Given that the vascular bundles are the main system of the material transportation (Apoplast and symplast) in the plants so the change in their structure can either disorder the water and the nutrient absorption of the soil or disturb the transfer of sugar and the photosynthetic products from the production location (Leafs) to the storage or consumption location. It seems that the lower vascular bundles surface in the first date of planting, regardless of the effect of the high temperature, is the mechanism to maintain the water and turgor pressure of the cells.

Table 4. Biennial mean comparison of anatomy characteristics flag leaf

Treatments	Large vascular bundles surface (μm^2)	Small vascular bundles surface (μm^2)	Xylem surface (μm^2)	Phloem surface (μm^2)
Sowing date				
D1	8704.324 *^{c}	1395.031 ^{a}	927.578 ^{a}	887.623 ^{b}
D2	10668.585 ^{a}	1602.902 ^{a}	1015.477 ^{a}	1211.051 ^{a}
D3	9268.74 ^{b}	1596.504 ^{a}	891.354 ^{a}	1149.941 ^{a}
Cultivar				
V1	9926.632 ^{a}	1600.199 ^{bc}	820.652 ^{cd}	954.178 ^{c}
V2	10058.754 ^{a}	1141.602 ^{d}	1236.764 ^{a}	913.758 ^{c}
V3	9519.857 ^{a}	1687.579 ^{ab}	922.075 ^{bc}	1448.684 ^{a}
V4	10078.784 ^{a}	1790.771 ^{a}	977.251 ^{b}	960.126 ^{c}
V5	8152.071 ^{b}	1437.245 ^{c}	767.292 ^{d}	1137.612 ^{b}

*Means in each column, followed by at least one similar letter(s) are not significantly different at 5% probability level using Duncan test.

D1, D2 and D3: Sowing dates 5 May, 26 May and 16 Jun, Respectively.

V1, V2, V3, V4 and V5: Hoveize, Hamar, Red Anbboori, Champa and Danial rice cultivars, respectively.

Table 5. Biennial mean comparison of anatomy characteristics flag leaf in experiment treatment

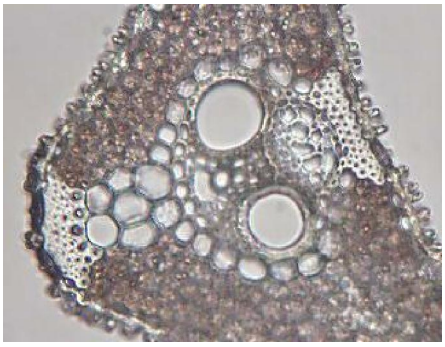
Sowing date	Cultivar	Large vascular bundles surface (μm^2)	Small vascular bundles surface (μm^2)	Xylem surface (μm^2)	Phloem surface (μm^2)
D1	**V1**	9331.317 *$^{d-f}$	1270.665 $^{d-f}$	950.587 $^{b-e}$	1073.438 ^{bc}
	V2	9699.553 $^{c-e}$	883.872 ^{g}	1057.183 $^{b-d}$	705.463 ^{de}
	V3	7223.355 ^{h}	1759.437 $^{a-c}$	879.563 $^{b-e}$	1063.017 ^{bc}
	V4	9633.790 $^{c-e}$	2013.780 ^{a}	1019.190 $^{b-d}$	955.865 $^{b-d}$
	V5	7633.603 ^{gh}	1047.403 ^{fg}	731.367 ^{e}	640.133 ^{e}
D2	**V1**	10762.762 ^{bc}	1505.933 ^{cd}	721.383 ^{e}	992.602 $^{b-d}$
	V2	11973.815 ^{a}	1430.740 $^{c-e}$	1521.650 ^{a}	1106.258 ^{bc}
	V3	11323.663 ^{ab}	1750.258 $^{a-c}$	961.882 $^{b-e}$	2085.238 ^{a}
	V4	10715.220 ^{bc}	1938.249 ^{a}	1094.228 ^{bc}	919.900 $^{b-e}$
	V5	8567.465 $^{e-g}$	1389.331 ^{de}	778.243 ^{de}	951.258 $^{b-e}$
D3	**V1**	9685.792 $^{c-e}$	2023.998 ^{a}	789.985 ^{de}	796.495 $^{c-e}$
	V2	8502.895 $^{e-g}$	1110.193 $^{e-g}$	1131.405 ^{b}	929.553 $^{b-e}$
	V3	10012.553 ^{cd}	1553.042 $^{b-d}$	924.780 $^{b-e}$	1197.798 ^{b}
	V4	9887.342 ^{cd}	1420.284 $^{c-e}$	818.333 $^{c-e}$	1004.613 $^{b-d}$
	V5	8255.143 $^{f-h}$	1875.002 ^{ab}	792.265 ^{de}	1821.245 ^{a}

*Means in each column, followed by at least one similar letter(s) are not significantly different at 5% probability level via Duncan test.

D1, D2 and D3: Sowing dates 5 May, 26 May and 16 Jun, respectively.

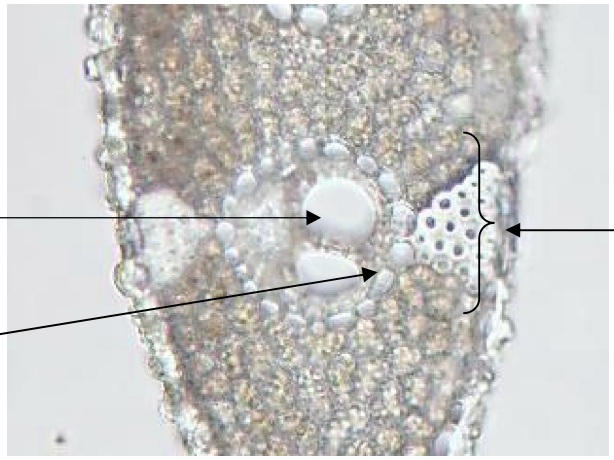
V1, V2, V3, V4 and V5: Hoveize, Hamar, Red Anbboori, Champa and Danial rice cultivars, respectively.

Because the number, diameter and the stomata surface of this date are smaller than of the other two dates, the transpiration is reduced, although this process somehow limits the entrance of CO_2 and reduces the photosynthesis. Among cultivars, regardless of the effect of temperature degree, gained result is mostly related to the genotypic differences, seeing in Danial, as a high yielding cultivar, that having smaller cells and thicker walls, lower rate of respiration, increase of photosynthetic yield, and water consumption are of the breeding purposes. Given the course of water movement in the plant and its exit from stomata, vascular bundles with smaller surface, because of having the longer distance from the inner surface of leaf epidermal cells (Place of water evaporation), transpiration is slower, which can be observed at the first date of planting (Figures 1, 2 and 3). The above mentioned concerning the role of vascular bundles on the rate of material allocation based on different planting dates are in consistent with other references (Rudall, 1994; Heywood, 1985). Small vascular bundles surface: results of compound analysis indicated that effects of year, cultivar, interaction effect of cultivar and year, date of planting, and simultaneous effect of three factors were significant at one percent probability level, but no statistically difference was observed for other items (Table 3). Although small vascular bundles the surface was in an identical class during all three dates, relatively, its amount during the second date was more than the other dates specially the first one. Among cultivars, Danial and Champa had the lowest and highest surfaces, respectively (Table 4). In the interaction effect of cultivar and date of planting, highest surface was belonged to the Champa at the first and second dates and Hoveyzeh at the third date (Table 5). Given that small bundles are mainly concentrated in the secondary veins radiating from the midrib, in addition to the surface, their number is also important. It seems that to achieve a more yield, a desirable rate of vascular bundles surface (Large and small) is needed because in developing a cell, although keeping the swelling pressure is too important, its realization depends on the movement of solvents or ions inside the cell; the availability of the sucrose as the main material transferable to the growing cells to synthesize the osmotic compounds and accumulation of dry matter is highly important. In the other hand, because of the necessary role of auxin hormone in the stimulating and softening cellulose micro fibrils and maintaining the swelling pressure, gained result should be found in the interaction effect between the chemical processes of the production and auxin accumulation as well as the accumulation of the solutes and physical effects of swelling pressure. Results concerning the effect of different planting dates on the small vascular bundles surface are in consistent with other studies` results (Metcalf and Chalk, 1950). Xylem surface: it was indicated in the present study that the effect of year, cultivar, interaction effects of the cultivar and year, simultaneous effect of all three factors are significant at 1% and interaction effects of planting date with year and cultivar are significant at 5 percent probability level (Table 3). Comparing averages, highest and lowest xylem surface is belonged to hamar at second planting date and Hoveyzeh plus Danial at the first date, respectively (Tables 4 and 5). Given that water movement in xylem, as well as being affected by cohesion force (Intermolecular absorption created by hydrogen link in water), is affected by water absorption through a solid phase (Wall of xylem).

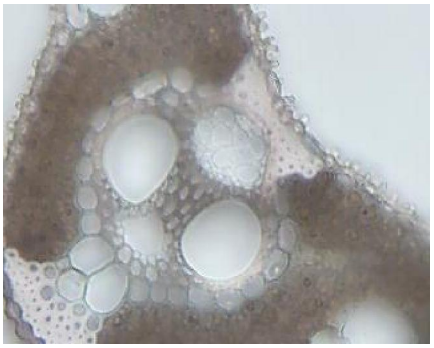


Hoveize cultivar

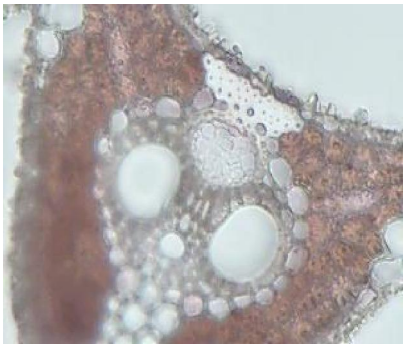
Hamar cultivar



Danial cultivar

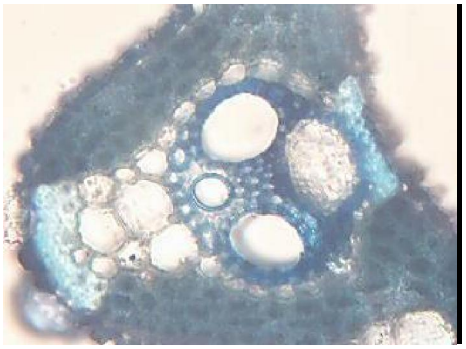


Champa cultivar

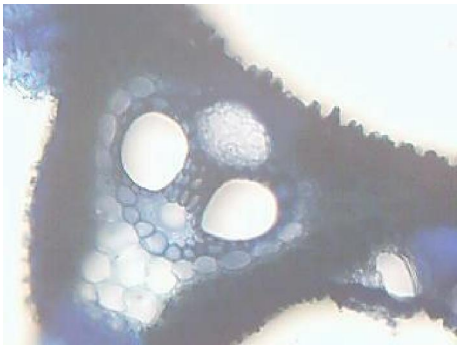


Red Anbboori cultivar

Fig 1. Anatomy flag leaf in first Sowing date

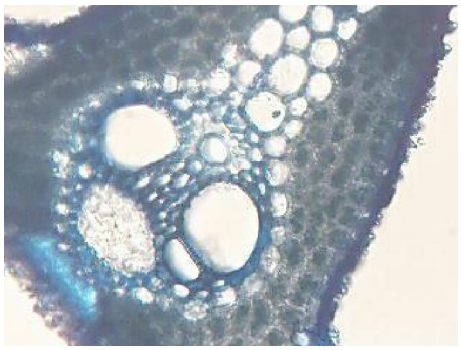


Hoveize cultivar

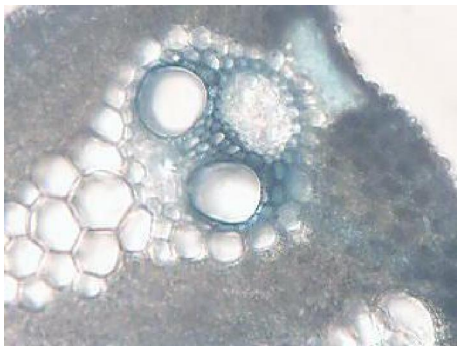


Hamar cultivar

Danial cultivar

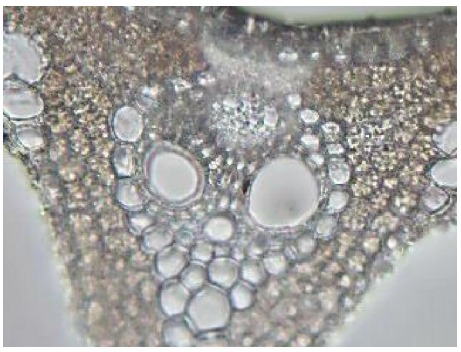


Champa cultivar

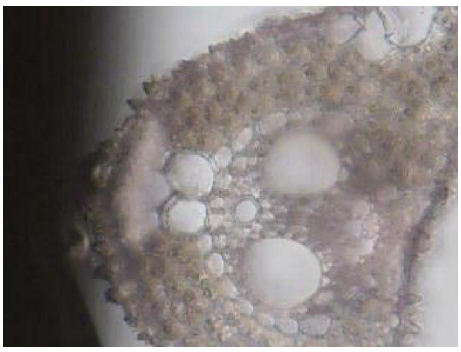


Red Anbboori cultivar

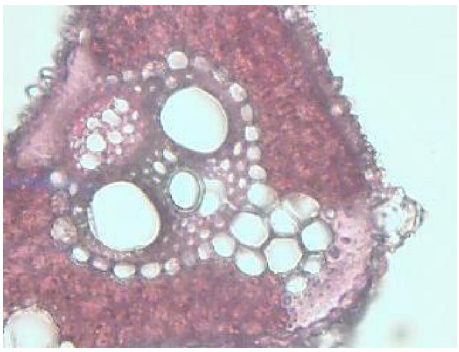
Fig 2. Anatomy flag leaf in second Sowing date



Hoveize cultivar

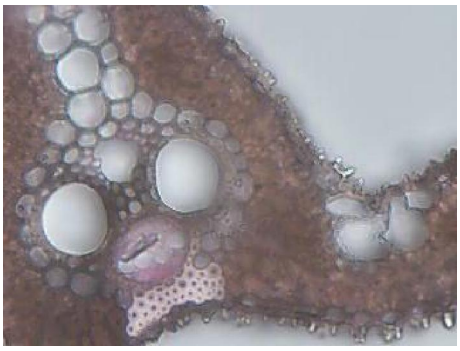


Hamar cultivar



Danial cultivar

Champa cultivar



Red Anbboori cultivar

Fig 3. Anatomy flag leaf in third Sowing date

with adhesion force, increase of the water capillary structure in the xylem structure depends on the contact angle of liquid and xylem wall, radius of xylem, and surface tension of the liquid in addition to the gravity force applied on it, because there is the diverse ratio between the rate of capillary increase in xylem and xylem radius structure so the smaller the xylem, the more the capillary force of keeping the water thus more pressure is needed to discharge the water from xylem. Consequently, in the Hoveyze and denial cultivars having smaller xylem, more force is applied for water preservation (Figures 1, 2 and 3). Results obtained with the report (Limouchi *et al.,* 2013) is consistent. Phloem surface: results of this study indicate that there is the significant difference at one percent probability level between planting date, cultivar, and interaction effect between the two and three factors (Table 3). Lowest phloem surface is belonged to the first planting date and among cultivars, in addition to the heat-tolerant cultivars, Champa had smaller phloem (Table 4). Depending on planting date, response of the cultivars was completely different seeing that Danial (In spite of its lowest surface at the first planting date) had the highest surface at the third planting date. In addition, highest surface of red Anbori and Danial cultivars were at the second and third dates, respectively (Table 5). Given that metabolic energy trend is needed to transfer sugar content in this path, reduction of phloem cell surface can predispose the material preservation and shortening the transfer path by reducing the rate of respiration and transfer intervals. In the other hand, size of phloem depends on the size of source and reservoir and their relationships (Figure 1, 2 and 3). Mentioned results similar with the report of Limouchi *et al.* (2013).

CONCLUSION

The results of combined analyses showed that was a significant different in planting date by variety interaction in all of trades. On the other hand, the related temperature condition, varieties had different reaction. Highest and lowest levels of large vascular bundles were related to 2nd and 1rd planting dates; but highest and lowest levels of small vascular bundles, xylem and phloem obtained in 2st and 1rd planting dates respectively.

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