Stereological Analysis of the Flag Leaf of Some *Triticum* L. Species

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Summary

Wheat genotypes from various Triticum L. genus species (*T. dicoccoides* Körn, *T. durum* Desf, *T. spelta* L. and *T. aestivum* L.) were analysed by stereological analysis in order to explore the existence of inter-species differences and similarities in anatomical characteristics of wheat flag leaf tissue. The genotypes of the tetraploid species did not differ significantly among themselves in the volume density of the analysed tissues of the flag leaf. Among the hexaploid species, the results showed highly significant differences in volume density of the photosynthetic tissue and volume density of the mechanical tissue; and significant differences for the volume density of the vascular tissue. The analysis of the main vein of the flag leaf showed smaller volumes of the vascular tissue in hexaploid species – of the phloem and parenchyma of the main vein. The analysed species showed the greatest difference in the volume of the main vein, the parenchyma of the main vein and the mechanical tissue. When compared to the volume and volume density of the xylem of the main vein, the tetraploid species had greater volumes and volume densities of the mechanical tissue of the main vein. With hexaploid species the results were reverse.

Key words: wheat, flag leaf, stereology

INRODUCTION

Despite already relatively good understanding of wheat, the interest in its origin, evolution and breeding is ever-increasing. The study of *Triticum* genus species is of special scientific and practical significance in developing new genotypes that give more yield than the existing ones, along with the utilization of positive characteristics of wild wheat as a starting point for the creation of new, better combinations (Pavićević, 1988). When assimilate transport and yield increase are concerned, what is specially important is the structural and functional organisation of the photosynthetic and vascular tissue (Kebede *et al.*, 1992). With *Triticum* species ploidy is accompanied by a change in the number, size and form of mesophyll cells. The longest multi-lobus cells were isolated in hexaploids, whereas in diploids the most numerous are cylindrical two- and four-lobus cells as well as irregularly shaped cells (Parker and Ford, 1982). With the increase of genome level, the maximum number of lobuses per cell increases, whereas the number of mesophyll cells and the frequency of one-lobus cells decrease (Sasahara, 1982). In the course of the evolution, the vascular tissue capacity has increased, measured as phloem area on the cross section of the upper part of the top node (Evans *et al.*, 1970). On the other hand, it is well known that in conditions advantageous to assimilate production and to high storage capacity, the number and size of vascular bundles, and especially of phloem area of the top node, limit productivity

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in wheat (Nátrová and Nátr, 1993). Bearing that in mind, stereological analysis performed in this research covers wheat genotypes of various species of Triticum genus, in order to study the existence of inter-species differences and similarities among anatomical characteristics of flag leaf.

MATERIALS AND METHODS

Stereological analysis of wheat flag leaf covered: *Triticum dicoccoides* Körn, *Triticum durum* Desf (genotype 1/93, pedigree Els 6406-61-4/5/B.Bal// 2 * Bye/Tc/3/Bye/Tace/4/Df 17-72073/44), *Triticum spelta* L. and *Triticum aestivum* L. (NS 032 genotype, pedigree NS 19-87/ Yugoslavia). Species *Triticum dicoccoides* Körn and *Triticum spelta* L. are autochthonous populations originating in Montenegro, and the genotypes of species *Triticum durum* Desf and *Triticum aestivum* L. are part of selection material of the Scientific Institute of Field and Vegetable Crops in Novi Sad. The plants were grown in a field environment with the application of usual field treatment. In the research the sample size was 5 leaves per genotype, taken during earing. The sampling of the leaf into 5 segments was done by systematic uniform method of random sampling for grass leaves (Kubínová, 1993). According to the results of Cruz-Olive and Weibel (1990) and Kubínová (1993), this sample size is considered sufficient for correct evaluation of the analysed structures. 20 μm cross sections of the leaves were made by parafin method and by sliding microtom, and dyed by safranin (Jensen, 1962; Blaženčić, 1990). Stereological analysis was performed by multipurpose test system with 42 test points (M 42).

The following values were calculated: leaf and main vein volumes, the volume and volume density of the epidermis, photosynthetic, vascular and mechanical tissues, as well as the phloem of the main vein bundle, xylem of the main vein bundle, parenchyma of the main vein and mechanical tissue of the main vein. All the given parameters were calculated based on Kubínová (1993). The data were processed by ANOVA for factorial experiments. The following were calculated: mean ($\bar{x}$), mean standard error ($S\bar{x}$) and variation coefficient (CV%). Mean difference relevance testing was done by t-test (*α =0.05 and**α = 0.01).

RESULTS

Stereological analysis of the flag leaf

The leaf volume of the analysed genotypes ranged from 375 mm^3 (*T. aestivum*) to 485 mm^3 (*T. spelta*), with a significant difference among all species, except between *T. durum* and *T. spelta*. In all observed species the greatest volume of the total leaf volume belongs to photosynthetic tissue (Tables 1, 2). The smallest volume of photosynthetic tissue was observed in *T. aestivum*, and the largest in T. *durum* and *T. spelta*. The results of the t-test show a highly significant difference in the volume of the photosynthetic tissue between *T. aestivum* cum *T. durum* on the one hand and *T. dicoccoides* on the other; and significant differences between *T. aestivum* cum *T. spelta* and *T. durum* cum *T. dicoccoides*. When epidermis and vascular tissue volumes are concerned, the species ranked the same, and the values ranged from 75 mm^3 to 102 mm^3 for epidermis volume and from 41 mm^3 to 74 mm^3 for vascular tissue volume. The comparison of the significance of mean differences showed that the species had highly significant differences in epidermis volume and vascular tissue volume at all levels of comparison. The exception is the observed absence of a significant difference in epidermis volume between *T. aestivum* and *T. dicoccoides*, and a significant difference of only $\alpha = 0.05$ in volume parenchima tissue between *T. durum* and *T. spelta*. Mechanical tissue volume exhibited similar values in all genotypes. A significant difference was obtained only between *T. spelta* and *T. dicoccoides*.

Table 1. Volume (V; mm^3) and volume density (V_v;%) of the flag leaf tissue in species of genus *Triticum*

Characters		**Tetraploids**				**Hexaploids**			
		T. dicoccoides		*T. durum*		*T. spelta*		*T. aestivum*	
		$\bar{x} \pm S\bar{x}$	CV	$\bar{x} \pm S\bar{x}$	CV	$\bar{x} \pm S\bar{x}$	CV	$\bar{x} \pm S\bar{x}$	CV
$V_{l.}$*	(mm^3)	441 ± 1.9	0.74	472 ± 2.9	1.08	485 ± 2.6	0.93	375 ± 1.0	0.47
$V_{e.}$	(mm^3)	79.0 ± 1.0	2.15	89.0 ± 0.2	0.39	102 ± 0.7	1.22	75.0 ± 0.3	0.63
$V_{ph.t.}$	(mm^3)	289 ± 0.4	0.26	302 ± 2.8	1.61	301 ± 4.3	2.48	250 ± 1.6	1.10
$V_{v.t.}$	(mm^3)	63.0 ± 0.6	1.66	71.0 ± 0.4	0.91	74.0 ± 0.6	1.55	41.0 ± 0.1	0.60
$V_{m.t.}$	(mm^3)	9.5 ± 0.1	2.58	9.4 ± 0.5	8.51	8.3 ± 0.3	7.23	9.3 ± 0.5	8.67
$V_{Ve.}$	(%)	18.0 ± 0.2	1.67	18.8 ± 0.1	0.53	21.1 ± 0.3	2.12	20.0 ± 0.1	1.01
$V_{V\,ph.t.}$	(%)	65.4 ± 0.2	0.53	64.0 ± 0.2	0.54	62.0 ± 0.5	1.53	66.6 ± 0.3	0.67
$V_{V\,v.t.}$	(%)	14.4 ± 0.09	0.98	15.1 ± 0.03	0.66	15.2 ± 0.23	2.63	10.9 ± 0.03	0.92
$V_{V\,m.t.}$	(%)	2.2 ± 0.03	4.55	2.1 ± 0.12	9.52	1.7 ± 0.06	5.88	2.5 ± 0.14	9.81

*l.-leaf; e.- epidermal tissue; v.t. – vascular tissue; ph.t. – photosyntetic tissue; p.t. – parenchyma tissue; m.t. – mechanical tissue

In analysed genotypes, out of the total leaf volume, the participation of mechanical tissue is from 1.7 % (*T. spelta*) to 2.5% (*T. aestivum*), vascular tissue from 10.9 % (*T. aestivum*) to 15.2 % (*T. spelta*), epidermis from 18.0 % (*T. dicoccoides*) to 21.1 % (*T. spelta*), whereas the greatest was photosynthetic tissue: 62.0 % (*T. spelta*) do 66.6 % (*T. aestivum*). The tetraploid species did not differ significantly in volume density of the analysed tissues. Among hexaploid species, highly significant differences were obtained regarding the volume density of the photosynthetic and mechanical tissues, and significant differences in the volume density of the vascular tissue. Species *T. aestivum* and *T. dicoccoides*, as well as *T. durum* and *T. spelta*, were significantly different only in the volume density of the epidermis. A significant, or a highly significant difference between *T. aestivum* and *T. durum* and also between *T. dicoccoides* and *T. spelta* was observed in the volume density of epidermis, photosynthetic and mechanical tissues. The variability of volume densities of the tissues, when compared to tissue volumes, was higher in most instances, and the variation interval ranged from 0.53% ($V_{Vph.t}$) to 9.8% ($V_{Vm.t}$).

Table 2. Difference significance in volume and volume density of the flag leaf in species of genus *Triticum*

		a:d	a:c	a:s	d:c	d:s	c:s
V_l	(mm^3)	**	**	**	**	ns	*
$V_{e.}$	(mm^3)	**	ns	**	**	**	**
$V_{f.t.}$	(mm^3)	**	**	*	*	ns	ns
$V_{p.t.}$	(mm^3)	**	**	**	**	*	**
$V_{m.t.}$	(mm^3)	ns	ns	ns	ns	ns	*
$V_{V\,e.}$	(%)	**	*	ns	ns	*	**
$V_{V\,f.t.}$	(%)	*	ns	**	ns	ns	**
$V_{V\,p.t.}$	(%)	ns	ns	*	ns	ns	ns
$V_{V\,m.t.}$	(%)	*	ns	**	ns	ns	*

a - *T. aestivum*; d - *T. durum*; c - *T. dicoccoides*; s - *T. spelta*

Stereological analysis of the main vein of the flag leaf

Stereological analysis showed that the values of the main vein volume ranged from 7.2 mm^3 (*T. spelta*) to 13.2 mm^3 (*T. durum*). In all the species, out of the total volume of the main vein, the vascular tissue – phloem had the smallest volume (0.35 mm^3 to 0.50 mm^3), and the main vein parenchyma had the largest volume (3.8 mm^3 do 7.6 mm^3). The tetraploid genotypes had larger volumes of mechanical tissue when compared to the volumes of the xylems, whereas with the hexaploids the situation was reverse. *T. spelta* and *T. durum* had lowest and highest values respectively of the following volumes: main vein, main vein parenchyma and mechanical tissue of the main vein (Table 3).

For all the above mentioned volumes, no significant difference was recorded between *T. aestivum* and *T. dicoccoides*, as well as between *T. aestivum* and *T. spelta*, only for the volume of the mechanical tissue of the main vein. Generally speaking, significant differences for most volumes were recorded between *T. durum* cum *T. aestivum* and *T. dicoccoides*, and between *T. dicoccoides* and *T. spelta*. The greatest variability of the analysed volumes was recorded in volumes of the phloem and mechanical tissue (Table 4).

Table 3. Volume (V; mm^3) and volume density (V_v;%) of the main vein of the flag leaf of species of genus *Triticum*

Characters	Tetraploids				Hexaploids			
	T. dicoccoides		*T. durum*		*T. spelta*		*T. aestivum*	
	$\bar{x} \pm S\bar{x}$	CV	$\bar{x} \pm S\bar{x}$	CV	$\bar{x} \pm S\bar{x}$	CV	$\bar{x} \pm S\bar{x}$	CV
Main vein* V	9.5 ± 0.1	2.11	13.2 ± 0.5	6.82	7.2 ± 0.1	1.96	9.5 ± 0.4	6.82
M.v. phloem bundle V	0.50 ± 0.03	6.32	0.39 ± 0.02	8.11	0.36 ± 0.02	21.5	0.35 ± 0.02	9.04
M.v. xylem bundle V	1.63 ± 0.1	8.68	2.10 ± 0.1	9.52	1.83 ± 0.1	1.71	1.93 ± 0.1	12.69
M.v. parenchyma V	5.4 ± 0.1	4.54	7.6 ± 0.2	5.26	3.8 ± 0.1	6.40	5.5 ± 0.3	10.91
M.v. mechanical tissue V	1.9 ± 0.06	5.26	3.0 ± 0.14	8.16	1.0 ± 0.07	14.10	1.7 ± 0.12	11.76
M.v. phloem bundle V_V	5.3 ± 0.3	9.40	3.0 ± 0.1	4.71	4.9 ± 0.3	11.20	3.7 ± 0.1	3.82
M.v. xylem bundle V_V	17.3 ± 1.0	10.45	16.0 ± 0.2	1.88	25.6 ± 0.1	0.55	20.2 ± 0.5	4.70
M.v. parenchyma V_V	57.3 ± 0.7	2.10	57.7 ± 0.5	1.39	52.5 ± 2.7	8.90	57.5 ± 1.6	4.70
M.v. mechanical tissue V_V	20.1 ± 0.1	0.70	23.3 ± 0.3	2.19	13.2 ± 0.7	5.70	18.6 ± 2.1	19.36

*Main vain- M.v.

Table 4. Significance of differences in volumes and volume densities of the main vein of the flag leaf of species of genus *Triticum*

	a:d	a:c	a:s	d:c	d:s	c:s
Main vein V	**	ns	*	*	**	*
M. v. phloem bundle V	ns	*	ns	ns	ns	*
M. v. xylem bundle V	*	*	ns	**	ns	ns
M. v. parenchyma V	**	ns	**	*	**	**
M. v. mechanical tissue V	*	ns	ns	*	**	*
Main vein phloem bundle V_V	ns	*	ns	*	*	ns
Main vein xylem bundle V_V	**	*	**	ns	**	*
Main vein parenchyma V_V	ns	ns	ns	ns	ns	ns
Main vein mechanical tissue V_V	*	ns	ns	*	**	*

a - *T. aestivum*; d - *T. durum*; c - *T. dicoccoides*; s - *T. spelta*

Out of the total volume of the flag leaf main vein, of all tissues, the phloem has the smallest percentage (3.0% - *T. durum* to 5.3% - *T. dicoccoides*). Regarding this tissue, *T. dicoccoides* was significantly different from *T. aestivum* and *T. durum*. As with the volumes, the tetraploid species had greater volume densities of the mechanical tissue, when compared to the volume densities of the xylems, whereas it was vice versa with the hexaploids. Highly significant differences in V_V of the main vein xylem bundle were obtained between *T. aestivum* cum *T. durum* and *T. spelta*, as well as between *T. durum* and *T. spelta*. When the percentage of the main vein mechanical tissue is concerned, *T. durum* had values significantly and highly significantly higher than other analysed species. There was a significant difference in V_V mechanical tissue in *T. dicoccoides* cum *T. spelta*. In the volume of the leaf main vein the main vein parenchyma had the greatest percentage (53.5% to 57.7%). The analysed species did not differ significantly in this tissue. The variability of volume densities of the analysed tissues is higher than the variability of their volumes.

DISCUSSION

The existence of great genetic variability, especially in close or distant relatives of hexaploid wheat, presents a basis for the introduction of new positive characteristics, created by a long evolution influence of the adequate genes, in interaction with the changeable conditions of the outside environment (Mac Key, 1984). On the other hand, genotypes with different morphological and anatomical characteristics of the leaf offer an opportunity for broad manipulation in further improvements of hexaploid wheat, and they can be useful in fast selection of genotypes for improved photosynthesis and lower respiratory level (Bhagwat *et al*, 1997). For further yield improvement, besides a better insight into genetic regulation of photosynthesis as a complex process, the availability of genetically variable material is also very important.

Quantitative analysis of the anatomical structure of the leaf is necessary for the study of the relationship between function and structure. The diffusion of CO_2 from the atmosphere through stomata to mesophyll cells, i.e. chloroplasts, is related to the organisation of the mesophyll, and above all to the number and volume of cells, as well as to the volume of intercellular space (Kubínová, 1994, Syversten *et al.*, 1995). The exposed mesophyll cell area determines the chloroplast area exposed to the diffusion of CO_2, so that intracellular diffusion greatly depends on the structural organisation of the mesophyll (Parkhurst and Mott, 1990).

Stereological analysis of the flag leaf showed the existence of highly significant differences in the volumes of the leaf of the genotypes of the analysed species, except in the case of comparison between *T. dicoccoides* and *T. spelta*, where there is only a significant difference. In all the analysed species, the photosynthetic tissue had the greatest volume out of the total leaf volume. In relation to the leaf volume, the genotype of species *T. aestivum* had a smallest volume of the photosynthetic tissue, while the genotypes of T. *durum* and *T. spelta* had the greatest. The results point to highly significant differences between *T. aestivum* cum *T. durum* and *T. dicoccoides* in the volume of the photosynthetic tissue, and significant differences *T. aestivum* cum *T. spelta*. The genotypes of tetraploid species did not differ significantly in the volume density of the analysed tissues. Among hexaploid genotypes highly significant differences were recorded in the volume density of the photosynthetic tissue and the volume density of the mechanical tissue, and significant differences in the volume density of the vascular tissue.

Comparing morpho-anatomical characteristics of the first leaf of the wheat genotypes of different levels of ploidy, Jellings and Leech (1984) found that hexaploids had a smaller number of mesophyll cells but of larger surface when compared to tetraploids, and that the percentage of the photosynthetic tissue and intercellular space per leaf area unit was higher than in hexaploid genotypes of wheat. The existence of longer multi-lobus cells with larger surfaces in hexaploid genotypes compensates for their smaller number than in tetraploids (Parker and Ford, 1982). Unlike these results, Kebede *et al.* (1992), searching for the explanation of the differences in photosynthetic activity between two genotypes (with similar leaf surface) of species *T.dicoccoides* and genotype of *T.aestivum*, found that there were no significant differences in anatomical structure, either within the same level of ploidy, or between different levels. The authors are of the opinion that the differences in photosynthetic activity of genotypes *T. dicoccoides* are caused by biochemical processes.

The anaysis of the main vein of the flag leaf of hexaploid species showed smaller volumes of vascular tissue – phloem and main vein parenchyma. Species *T. spelta* and *T. durum* had lowest and highest values, respectively, for most characteristics. The analysed species differed most in the volume of the main vein, main vein parenchyma and mechanical tissue. In the total volume of the main vein of all analysed genotypes, of all tissues, the lowest density is that of the phloem, and the greatest that of the main vein parenchyma. Tetraploid species had greater volumes and volume densities of the main vein mechanical tissue when compared to the volumes and volume densities of the main vein xylem. The hexaploids exibited inverse values. Greater volume density of the main vein xylem in hexaploid species genotypes, points to their need to transport larger quantities of inorganic matter.

As the form and size of the mesophyll cells depend also on the conditions of the environment (especially light) in which the leaves develop, some authors believe that this inter-species variability is a result of different adaptability (Friend and Pomeroy, 1970; Khan and Tsunoda, 1970).

The results of this research confirm that ploidy level is reflected onto the structural organization of the leaf. If the experiment would be performed in semicontroled laboratory conditions, with a higher level of confidence, it may be stated that the differences in structural characteristics of lamina leaf genotypes of different ploidy level are caused by ploidity. Such an experiment is planned in the future research.

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