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Title: Experimental Studies on Species Relationships in T. turgidum ssp. carthlicum and T. aestivum ssp. carthlicoides

Abstract approved: Robert J. Metzger

A study of populations characteristics of T. turgidum ssp. carthlicum and T. aestivum ssp. carthlicoides was completed using numerical analysis. A sample of 74 accessions of ssp. carthlicum and 20 of ssp. carthlicoides were scored for 46 characters. It was established that three morphological distinguishable groups of ssp. carthlicum can be recognized. These data provide supportive evidence for the recognition of var. rubiginosum Zhuk. and var. fuliginosum Zhuk. as distinct taxa. However, recognition of var. stramineum Zhuk. based on spike color could not be achieved. Variation patterns in ssp. carthlicum suggests that geographical distance and taxonomic distance between populations are associated. Also, the numerical analysis showed that a combination of characters could be used to separate T. aestivum ssp. vulgare from ssp. carthlicoides. These results provided justification to regard the latter as a separate taxa at the level of subspecies, namely T. aestivum ssp. carthlicoides.

The phylogenetic relationship between T. turgidum ssp. carthlicum and other T. aestivum subspecies was examined based on SDS-PAGE analysis of the HMW glutenin subunits. The 74 carthlicum

accessions gave identical HMW glutenin subunits profile, indicating a monophyletic origin of this subspecies. The lack of variability also suggests its recent origin. This interpretation seems to favor the hypothesis that ssp. carthlicum is a young form derived from a cross between a Q-bearing hexaploid and a tetraploid wheat. The similarity in the profile presented by ssp. carthlicoides and ssp. vulgare indicated that either form could be the hexaploid parent in the cross that led to the formation of carthlicum. The removal of the D genome from vulgare cultivars, however, resulted in an HMW glutenin profile which closely resembles that of ssp. carthlicum, but is substantially different from that of the original hexaploid cultivars. These results confirm the close relationship which appears to exist between aestivum and carthlicum. Also, the influence of the D genome on the differential expression of HMW glutenin subunits demonstrates the limitations of interpreting evolutionary events based on the concept of simple additive relationship between genomes and protein subunits.

EXPERIMENTAL STUDIES ON SPECIES RELATIONSHIPS IN *T. TURGIDUM* SSP. *CARTHLICUM* AND *T. AESTIVUM* SSP. *CARTHLICOIDES*

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DEDICATION

My wife, Maria, and our children for their help, patience, and sharing of the burdens of completing a graduate program. And, to my parents I am grateful for providing the initial opportunity to go to college some years ago.

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INTRODUCTION

The history of plant systematics, particularly that of cultivated plants, clearly illustrates attempts to arrange in a satisfactory system all morphological, physiological, and cytological characteristics that can be helpful in the classification of plant specimens in different regions. In the specific case of wheat, interest in its evolution arose not only due to the great diversity of forms, but also to the fact that these processes have been influenced to a great extent by man.

Several taxonomic arrangements of the genus *Triticum* have been proposed by a number of researchers (Percival, 1921; Schiemann, 1951; Bowden, 1959; Mac Key, 1966, 1968; Morris and Sears, 1967; Dorofeev et al., 1979; Love, 1984). Yet, the evolutionary phases and a satisfactory taxonomical classification of the genus *Triticum* have not been fully elaborated. Nomenclature in the wheat group is still not uniform in the literature and constant proposed changes and modifications continue to enlarge the confused taxonomic literature. Variability within species and human intervention account for the great difficulty in systematization and lack of harmony in this taxonomic group.

One of the primary purposes of this investigation was to determine the role *T. turgidum* ssp. *carthlicum* may have played in the origin and evolution of *T. aestivum*. This cultivated tetraploid

subspecies has a restricted distribution in the Soviet Socialist Republics of Dagestan, Georgia, and Armenia, and northeastern Turkey; the same area where hexaploid types are considered to have originated (Vavilov, 1960, 1981). Subspecies carthlicum is the only tetraploid in the genus Triticum which has the free-threshing and rachis toughness characteristics controlled by the same inhibiting system, Q-factor, that operates in all free-threshing hexaploid forms (Mac Key, 1966). Due to the presence of the Q-factor, ssp. carthlicum is very similar in appearance to some forms of T. aestivum (Vavilov, 1960; Kuckuck, 1979). This similarity and the fact that carthlicum is often found growing in mixed populations with T. aestivum ssp. carthlicoides suggest a close relationship between these forms. The close similarity between ssp. carthlicum and ssp. carthlicoides has caused misclassifications by early botanists and breeders. Vavilov (1960) pointed out the close resemblance between the tetraploid and hexaploid forms, and the difficulties in discriminating them. Glume awn length was considered the most reliable character for distinguishing between these two forms. However, a study of the morphological variation patterns within ssp. carthlicum and ssp. carthlicoides specimens, using discriminant analysis, showed the length of glume awns alone cannot be used to distinguish between these two forms (Vieira, 1984).

T. turgidum ssp. carthlicum was previously considered to be relatively homogeneous ecologically (Dekaprelevich, 1923), but additional studies of this taxon showed a certain degree of

diversity (Zhukovski, 1923; Gandilyan, 1976). Regarding T. aestivum ssp. carthlicoides, the ranks assigned to this taxon differ among the taxonomic treatments. Gandilyan (1976) recognized it as a convariety. At the other extreme, Kuckuck (1979) recognized this form as a subspecies.

The aims of this investigation were to increase the present knowledge of the origin and evolutionary pathways of these two taxa. Thus, in this study, we used the association of morphological, numerical, cytological, and chemical analytical procedures to investigate these subspecies.

MANUSCRIPT 1

A NUMERICAL ANALYSIS OF *T. TURGIDUM* SSP. *CARTHLICUM* AND *T. AESTIVUM* SSP. *CARTHLICOIDES*

ABSTRACT

The present investigation reports the results of numerical analysis of T. turgidum (L.) Thell. ssp. carthlicum (Nevski) Love et Love and T. aestivum (L.) Thell. ssp. "carthlicoides". A sample of 74 accessions of ssp. carthlicum and 20 of ssp. carthlicoides were scored for new morphological characters and re-examined for traditional characters. It was established through cluster and discriminant analysis that three morphological distinguishable groups of ssp. carthlicum can be recognized. These data provide supportive evidence for the recognition of var. rubiginosum Zhuk. and var. fuliginosum Zhuk. as distinct taxa. However, recognition of var. stramineum Zhuk. based solely on spike color could not be achieved. Variation patterns in ssp. carthlicum suggests that geographical distance and taxonomic distance between populations are associated. Further research is suggested before formal categorization of the ssp. carthlicum group is undertaken. Also, the numerical analysis showed that a combination of characters could be used to readily separate T. aestivum ssp. vulgare from the "carthlicum-like" hexaploid forms. These results provided justification to regard the latter as a separate taxa at the level of subspecies, namely T. aestivum ssp. carthlicoides.

Key words: numerical analysis, Triticum, subspecies, taxonomy, genetic diversity.

INTRODUCTION

The genus Triticum is well known for its taxonomic complexity at all levels. Although it contains some species that are clearly characterized, others grade into one another and can be isolated only with difficulty.

It is generally accepted that bread wheat is an amphiploid containing three complete sets of paired chromosomes (A, B, and D), inherited from three diploid progenitors, all of which probably evolved from one common ancestor. At present, it is not certain which tetraploid species was involved in the origin of hexaploid wheat, and how the genomes of the progenitor species have changed during evolution. Sears (1956) suggested three possibilities for the origin of free-threshing hexaploid wheats: a) through a cross between T. turgidum ssp. carthlicum x Ae. squarrosa; b) result of segregants of a cross between T. aestivum ssp. spelta x T. turgidum ssp. carthlicum, and c) as a result of mutation in T. aestivum ssp. spelta. Several papers have been published favoring one or another hypothesis (Tanaka, 1959; Kihara et al., 1965; Mac Key, 1966).

These hypotheses suggest ssp. carthlicum may hold an important position in the phylogeny process in the genus Triticum. The consideration that T. turgidum ssp. carthlicum receives as a possible progenitor of the free-threshing hexaploids derives from the fact that it is the only tetraploid that possesses the Q-factor (Mac Key, 1966). At the hexaploid level, all free-threshing forms are based on the presence of the Q-factor. Also, another element that brings controversy regarding the role played by carthlicum in

the evolutionary pathways of the hexaploid wheat is its restricted distribution in the Caucasus Region - Soviet Socialist Republics of Dagestan, Georgia, Armenia and northeastern Turkey.

Due to the presence of the Q-factor, carthlicum closely resembles some forms of common hexaploid wheat in spike morphology and kernel characteristics ("carthlicum-like" hexaploid forms). In his analysis of the flora of the Caucasus, Vavilov (1960) pointed out the close similarity in appearance between carthlicum and certain forms of aestivum which he classified as T. vulgare gr. persicoides. Kuckuck (1979) proposed that this taxon should be considered the parental form from which genes for spike morphology, together with the Q-factor, were transferred to ssp. carthlicum. He suggested the denomination T. aestivum ssp. carthlicoides for this hexaploid form. Gandilyan (1976) like Kuckuck separated this form based on the length of the glume awns, and proposed the denomination T. aestivum convar. tetraristatum.

T. turgidum ssp. carthlicum has been previously separated in two ecologically distinct groups: f. caucasionis dika and f. dzhavachetica dika (Dekaprelevich, 1925). The taxonomic treatment of carthlicum proposed by Zhukovski (1923) recognized four varieties within this taxon: var. stramineum, var. rubiginosum, var. coeruleum, and var. fuliginosum.

The purpose of this study was to use numerical analysis to examine the genetic diversity within ssp. carthlicum and compare the results with the conventional taxonomic separation of individuals. Regarding ssp. carthlicoides, the scope of this report was to verify

whether this form could be singled out from a population of T. aestivum and to assess the relative importance of all characters for its classification. The data were analyzed with cluster and discriminant analysis.

MATERIALS AND METHODS

Experimental Material

The specimens upon which this study was based were divided into three groups:

1. Specimens collected at random from 12 populations across northeastern Turkey during a germplasm collection expedition (TK collection) made by R.J. Metzger, J.A. Hoffmann, H.A. Sencer, and M. Kanbertay in 1979.
2. The second set of OTU's (Operational Taxonomic Unit) was composed by all known T. turgidum ssp. carthlicum accessions in the Small Grain Collection of U.S. Department of Agriculture.
3. The third group consisted of specimens reselected from the original TK or U.S.D.A. collections due to differences in morphological characteristics from the main form.

All entries were previously classified as T. turgidum ssp. carthlicum or T. aestivum ssp. carthlicoides based mainly on the glume awn length.

Character Observations

Thirty-one quantitative and fifteen qualitative characters were measured or coded at the same growth stage in fresh material (Tables 1.1, 1.2). The characters were determined in each of four greenhouse established plants for each accession. Vegetative and generative characters were always studied on the plants main stem.

Determination of glume characteristics was confined to the center 1/3 of the spike, with the exception of glume awn length.

Generally, awn length increased from the spike base to its apex. For this reason, measurements on the basal, middle, and penultimate spikelets were used to assess this character. Spike density was determined using the index suggested by Percival (1921). Degree of stem solidness was assessed using the scale of Larson (1959) in three cross sections of the uppermost internode, and one cross section in the middle of the second and third internodes. To examine leaf pubescence, the middle section of the fifth leaf was cleared in a fluid composed of lactic acid (85%), phenol, clove oil and xylene in proportions of 2:2:2:1, by weight (Herr, 1971), and the number of hairs in 4.0 mm^2 was counted using a high contrast Panasonic TV monitor attached to a Zeiss microscope via a Dage video camera. Chromosome number was determined using root tip cells.

Statistical Analysis

Multivariate numerical methods were used to perform the taxonomic analysis of the complex. Specimens were grouped by ploidy level resulting in two data matricies: 1) HEXA - composed of ssp. vulgare and ssp. carthlicoides accessions and 2) TETRA-tetraploid forms assigned as T. turgidum ssp. carthlicum.

The numerical analysis were conducted in two stages. In the first stage, cluster analysis was used to examine the distribution of the OTU's. The program used for the cluster analysis, CLUSB (made available by Dr. C.D. MacIntire, Department of Botany and Plant Pathology, Oregon State University) is a devisive, non-hierarchical clustering program which breaks a group of specimens into

small sets of clusters, minimizing at each stage the total within-cluster variance.

In the second stage, the stepwise discriminant analysis subprogram of BMDP 7M (Jennrich and Sampson, 1981) was used to compare the hypothesized population produced by the CLUSB program. Besides classification per se, discriminant analysis was used to determine those variables that are important in discriminating the groups, and the influence of each variable in deciding how individuals were classified.

The discriminant analysis procedures used in this investigation were based on the steps recommended by Sanathanan (1975):

1. Selection from a tentative list of variables, a number that contain most of the classificatory data;
2. Ordainment of the discriminant functions of the select variables;
3. Identification of features that represent major group differences; and
4. Final classification.

Multivariate techniques are thoroughly presented in Sneath and Sokal (1973), and information regarding their use in morphological studies is found in Dancik and Barnes (1975).

RESULTS

Morphological Variation in *T. turgidum* ssp. *carthlicum*

The validity of any numerical analysis is a function of the number and diversity of the characters included in the analysis. Consequently, an effort was made to examine the morphological variation as objectively as possible by selecting a group of traits which would reflect a great number of features of the plants and measuring these in a standard fashion for each OTU.

In the first stage of the numeral analysis of the cluster program, CLUSB, was used to establish objective and phenotypically homogeneous groupings. Morphological data of 46 variables measured on each of 74 specimens were used in this stage (Tables 1.1, 1.2). This clustering program tended to group the specimens into two or three relatively distinct clusters and a variable number of more diffuse and overlapping clusters. Although this program minimizes within cluster sum-of-squares at each step, there is a tendency to bring the groups closer together in hyperspace at higher clustering levels, so it may result in clusters that are barely distinct. With the data arrangement used here, at high clustering levels the program divided relatively homogeneous groups into arbitrary sub-clusters, and many individual specimens were isolated into single membered clusters. Less stable groups were isolated by comparing runs using different number of characters and OTU's. Three groups of specimens consistently clustered together in all runs, with a fourth cluster being formed by a single-membered OTU. This outlying cluster was removed and the clustering was repeated, in order to

make clear the pattern formed by the remaining first three groups. OTU's used in this first stage analysis were arranged in three groups denominated groups A, B, and C, and consisted of 31, 30, and 12 OTU's, respectively.

In the second stage of the numerical analysis, the discreteness of these three groups was examined by stepwise discriminant analysis, which is a multivariate technique used to compare two or more populations. Character variability among the populations is compared with that within populations. Generally, an accession is assigned to the group whose centroid is close to the individual. Discriminant analysis might be used to attempt to clearly define clusters, but the problem of assignment to groups becomes a problem that may lead to circularity (Neff and Smith, 1979). To avoid the circularity inherent in group circumscription and subsequent discrimination based on the same variables, only 28 characters, out of the initial 46 used in forming the groups by the CLUSB program, were utilized in this stage (Tables 1.1, 1.2).

Seven morphological features were selected in the stepwise discriminant analysis of the TETRA group (Table 1.3). Characters which showed the highest power of discrimination among these three groups were stem height, stem solidness-middle of the second internode, second leaf width, spike density, glume awn length-spike base, rachis hair position and spike color at maturation.

One measure of success of any discriminant procedure is to calculate the percentage of misclassification which can be expected when classifying individuals. The BMDP 7M program provides a direct

and jackknifed classification of all individuals. Jackknifing is a method in which the analysis is repeated, omitting one sample at a time and classifying that sample to the group for which it has the highest probability of membership. In this classification procedure, the overall percentage of the correctly classified specimens was 91.7%.

Certain OTU's were designated as having been misclassified in the discriminant analysis. It should be noted here, however, that in all cases, the initial classification of the specimens appears to have been correct. The apparent misclassifications generally resulted from the exclusion of variables used in the first stage from the analysis, or from marginal values for one of the variables. In this latter case, the specimens were otherwise typical of the group.

Figure 1.1 presents the plot of OTU's in the discriminant space by the canonical variates. The first canonical variate accounts for 82.6% of the total variation in the data matrix.

Table 1.4 displays means, standard deviation, and ranges of the 7 characters which were chosen by discriminant analysis to assign the specimens in groups A, B, and C. Spike density appears to be a useful character to separate groups B and C. Groups A and C showed a very similar distribution (Fig. 1.2). Glume awn length was used by other investigators to differentiate between ssp. carthlicum and other Triticum forms (Percival, 1921; Zhukovski, 1923; Kuckuck, 1979). However, quantitative analysis of this trait among accessions of ssp. carthlicum has not been reported. Specimens composing

group B tended to present longer awns than the other groups. In spite of similar distribution among all three groups, accessions of the groups A and C seems to concentrate in the lower end of the range (Fig. 1.3). Also, the same distribution pattern in all groups was observed for lemma awns, indicating a possible association among these traits (data not shown).

T. turgidum ssp. carthlicum is characterized by its degree of stem solidness. It represents one of the most useful characteristics for discriminating this form from ssp. carthlicoides (Vieira, 1984). Also, this feature was useful in infraspecific discrimination mainly between groups A and B (Table 1.4). Second leaf width displayed a high power of discrimination among the three clusters (Table 1.4). The only two qualitative characters that provided useful information about group variability were rachis hair position and spike color at maturation. All OTU's composing group B were characterized by having hair at rachis nodes and internodes (Fig. 1.4). Color of spike at maturation was the most reliable character to isolate group C. Spikes of all specimens in this group were black with white background (Fig. 1.5). Following the general tendency observed in other characters, group A showed the greatest variation for color of spike.

Morphological Variation in T. aestivum ssp. carthlicoides

To determine overall pattern of variation within the HEXA group, a data set of 30 OTU's was analyzed by the same methods used for TETRA group. Cluster analysis using CLUSB, with all 46 charac-

ters, showed three clearly distinct clusters, which were denominated groups 1, 2, and 3. These groups were composed of 20, 6, and 4 specimens, respectively.

For the assignment of individuals to different groups with discriminant analysis, only 27 characters were used (Table 1.1, 1.2). Six traits were selected by the stepwise discriminant program: glume awn length-spike top, spike density, hair density-abaxial surface, number of internodes, first internode length, and keel teeth position (Table 1.5). These six traits were used to separate ssp. carthlicoides from ssp. vulgare. In jackknifed classification procedure, 100% of OTU's were correctly identified as to their respective group source.

Figure 1.6 displays the ordination of these three groups in two dimensions of canonical space. The first canonical variate summarized 87% of the variation in the data. Table 1.6 presents the means, standard deviation and ranges of the six characters which showed the highest power of discrimination among the three groups. The characters used in computing the linear classification function (Table 1.5) were chosen in a stepwise manner. Glume awn length-spike top was the character which maximized the most the overall multivariate F ratio for test differences among specimens means. Glume awn length has been referred to by Gandilyan (1976) and Kuckuck (1979) as the pivotal character to isolate ssp. carthlicoides from the usual short glume awn forms of T. aestivum. The inspection of character differences between groups showed that OTU's of group 1 have distinctly longer glume awns than the other two.

The lowest observed value for this character in group 1 (3.0 cm) is considerable higher than groups 2 and 3 upper limit values (Table 1.6; Fig. 1.7). Also, the difference in leaf pubescence is a readily distinguishable morphological character acting to cause separation of group 1 from the others. OTU's composing group 1 are characterized by having greater hair density on the leaf surface (Table 1.6; Fig. 1.7). These differences indicate that the numerical analysis used in this study could clearly isolated ssp. *carthlicoides* from other forms.

DISCUSSION

The objectives of this investigation were to assess the genetic diversity within ssp. carthlicum and ssp. carthlicoides, and to identify additional characters for those groups. To achieve these goals, data on a variety of morphological traits were examined. The characters used included those that have been used previously in the taxonomy of these groups and others suggested by the literature or resulting from our observations.

The results of the numerical analysis indicate clearly that population differentiation has occurred within ssp. carthlicum. Plants can be easily grouped into three morphologically distinct clusters. Although often in agreement with the accepted conventional taxonomic separation into varietal rank, certain differences were observed between the numerical approach here used and the conventional analysis presented by Zhukovski (1923). Agreement was good between the two approaches regarding groups B and C. The specimens composing these two groups adhere to the original description of var. rubiginosum and var. fuliginosum recognized by Zhukovski (1923). The differences between the two types are principally based on the color of the spike at maturation, leaf width, and stem height. Variation observed within these two groups is believed to be genotypic, and it may also be suggested that the grouping pattern reflects some spatial distribution. Group B is composed almost exclusively of accessions collected in the Kars region in Turkey; whereas, accessions making up group C have a restrict distribution on the southern slopes of the main Caucasus

Mountains, mainly Georgia and Dagestan in the Soviet Union (Vavilov, 1926). These populations have formed separated clusters indicating the parallelism between geographical distance and genetic diversity in carthlicum. This association has also been observed by Dekaprelevich (1925) who stated that black-eared forms represent mountainous-forest ecotype, distributed mainly in the zone of 900-1400 m, whereas red or white-eared forms are characteristic from mountainous-steppe habitats at higher elevations - 1400 to 2100 m. The variability observed in these two groups is probably in great part due to their differential adaptation to water stress. Reduced leaf area, longer awns, and higher hair density on the leaves observed in var. rubiginosum are clear examples of morphological features found to be closely associated with the ability of the plant to endure drought induced stress (Stalfelt, 1959; Kramer, 1959; Grundbacher, 1963).

Accessions composing group A exhibited considerable polymorphism. This group showed close affinity in many characters to group B. Notable differences were the decreased length of the glume awn and degree of stem solidness, and the presence of some accessions with white spike (Table 1.4). Forms with white spike have been observed to fit var. stramineum as described by Zhukovski (1923). However, it is doubtful that this single character is diagnostic as in the case of black glumes var. fuliginosum. It was demonstrated by the discriminant analysis that reliable resolution of this group is only possible when morphological characters are used in combination. Probably this group also represents an ecotype. The characters which differed from the other groups were

those showing plasticity and may be related to habitat differences (Table 1.4). The possibility that this group represents the result of natural hybridization of var. rubiginosum and var. fuliginosum, or represents an original undifferentiated entity from which the other two arose cannot be excluded. The extremes shown in character expression by this group may be the main cause for difficulty in identification of such individuals by means of readily distinguishable characters. However, the extremes in expression did not prevent the discrimination of a group by the numerical analysis used; and, in fact, may have been responsible for their separation. These results suggested the need for a much closer examination of the ssp. carthlicum to reassess the characteristics used in classifying infraspecific taxa.

Several characters contributed to produce a sufficiently stable picture of overall variation, as well as achieving the greatest degree of discrimination of T. aestivum ssp. carthlicoides specimens. Glume awn length can be used to readily separate ssp. carthlicoides from ssp. vulgare. In spite of the importance of leaf pubescence to characterize a taxon (Percival, 1921), no reference to the value of this trait in the taxonomy of ssp. carthlicoides can be found in the literature. In the present study, however, the variation found for hair density on the leaf blade is meaningful in discriminating between ssp. carthlicoides and ssp. vulgare (Table 1.6).

Our results clearly demonstrate that ssp. carthlicoides and ssp. vulgare are two distinct morphological taxa. The support for

this isolation is evident since all ssp. carthlicoides specimens were contained in a single group after cluster analysis, and 100% probability of accurate delineation of this form was achieved when discriminant analysis was used.

The concept of subspecies proposed by Rothmaler (1955) offers some arguments in favor of subspecific rank for this taxon. For him, a subspecies is a group of plants more or less separated by a combination of several small and usually quantitative differences, but rarely isolated genetically. The results of the numerical analysis revealed that ssp. carthlicoides and ssp. vulgare can be separated from each other by certain morphological characters used in combination, as is the case of discriminant function (Table 1.5). Two characters, glume awn length and hair density provided the most reliable information for identification of each group, and without doubt contributed to satisfy the "75% rule" mentioned by Mayer et al. (1953). Additionally, the restricted distribution of carthlicoides in the area in which carthlicum is also found (Vavilov, 1926) and its importance in understanding the history and evolution of T. turgidum ssp. carthlicum (Kuckuck, 1979) clearly demonstrate that this form merits recognition as a separate subspecie. The process of establishing the nomenclature of this subspecies with corresponding synonymy and typification will be performed.

TABLE 1.1. Quantitative characters used for numerical analysis

(1) No.	(2) No.	(3) No.	Characters	(4) Acronym	Units of measurement
1	1	1	Days to heading	HDDT	Days
2	2	2	Stem height	STHT	cm
3			Stem thickness	STMT	cm
4		3	Number of internodes	NONO	--
5		4	First internode length	INTL	cm
6	3	5	Stem solidness - 2.5 cm below spike base	STMA	Index
7	4	6	Stem solidness - middle of first internode	STMB	Index
8	5	7	Stem solidness - 2.5 cm above first node	STMC	Index
9	6	8	Stem solidness - middle of second internode	STMD	Index
10	7	9	Stem solidness - middle of third internode	STME	Index
11	8	10	Flag leaf length	FLLL	cm
12	9		Flag leaf width	FLLW	cm
13	10	11	Second leaf length	SLLL	cm
14	11		Second leaf width	SLLW	cm
15	12	12	Third leaf length	TLLL	cm
16	13		Third leaf width	TLLW	cm
17	14	13	Hair density - abaxial surface	UPHD	--
18	15	14	Hair density - abaxial surface	LOHD	--
19	16	15	Spike length	SPKL	cm
20			Number of spikelets spike	SPSP	--

Table 1.1 (continued)

(1) No.	(2) No.	(3) No.	Characters	(4) Acronym	Units of measurement
21	17	16	Spike density	SPKD	Index
22			Glume length	GLML	cm
23			Glume width	GLMW	cm
24			Hair length - abaxial surface	UPHL	mm
25			Hair length - abaxial surface	LOHL	mm
26	18	17	Glume awn length - spike base	GLPS	cm
27	19	18	Glume awn length - spike middle	GLPM	cm
28	20	19	Glume awn length - spike top	GLPT	cm
29	21	20	Lemma awn length - spike base	AWLB	cm
30	22	21	Lemma awn length - spike middle	AWLM	cm
31	23	22	Lemma awn length - spike top	AWLT	cm

(1) Characters included in the first stage of the numerical analysis.

(2) Characters included in the second stage of the numerical analysis of Triticum turgidum ssp. carthlicum

(3) Characters included in the second stage of the numerical analysis of the hexaploid forms

(4) Acronyms for characters included in the discriminant analysis.

TABLE 1.2 Qualitative characters used for numerical analysis.

(1) No.	(2) No.	(3) No.	Character	(4) Acronym	Coding states
1			Leaf color at heading	LFCH	(1) Glaucous
					(2) Green
					(3) Dark green
2		1	Spike color at heading	SPCH	(1) Glaucous
					(2) Light green
					(3) Green
3	1	2	Spike color at maturation	SPCM	(1) White
					(2) Light red
					(3) Dark red on top of glume and light red on the base
					(4) Dark red
					(5) Black with white background
					(6) Brownish gray
4			Anther color	ANTC	(1) Yellow
					(2) Purple
5			Glume consistency	GLMC	(1) Ordinary
					(2) Papery
6			Keel size	KLSZ	(1) Narrow
					(2) Wide

Table 1.2 (continued)

(1) No.	(2) No.	(3) No.	Character	(4) Acronym	Coding states
7			Keel development	KLDV	(1) Entire glume (2) Not reaching base of the glume
8		3	Keel dentation	KLDT	(1) Almost no teeth (2) Few (3) Intermediate (4) Abundant
9		4	Keel teeth position	KLTP	(1) Only in the upper part of the glume (2) Reaching the middle of the glume (3) Entire glume
10			Glume shoulder shape	GLMS	(1) Almost lacking (2) Bevelled (3) Raised (4) Almost straight
11	2	5	Hair of the rachis	RACH	(1) Abundant (2) Intermediate (3) Slightly hairy
12	3		Rachis hair length	RACL	(1) Long

Table 1.2 (continued)

(1) No.	(2) No.	(3) No.	Character	(4) Acronym	Coding states
					(2) Intermediate
					(3) Short
13	4		Rachis hair position	RACP	(1) At sides and base of spikelet
					(2) At nodes and internodes
14			Caryopsis color	CARC	(1) White
					(2) Light red
					(3) Red
15	5		Caryopsis shape	CARS	(1) Short - narrow
					(2) Short - broad
					(3) Long - narrow
					(4) Long - broad

(1) Characters included in the first stage of the numerical analysis.

(2) Characters included in the second stage of the numerical analysis of Triticum turgidum ssp. carthlicum.

(3) Characters included in the second stage of the numerical analysis of the hexaploid forms.

(4) Acronyms for characters included in the discriminant analysis.

TABLE 1.3. Variables selected by the stepwise discriminant analysis and classification functions for the TETRA groups

		Classification functions		
Acronym	Description of variable	Group A	Group B	Group C
STHT	Stem height	1.41	1.16	1.33
STMD	Stem solidness - middle of 2nd internode	4.00	8.84	6.93
SLLW	Second leaf width	136.16	119.71	143.99
SPKD	Spike density	10.11	7.72	9.16
GLPD	Glume awn length - spike base	-8.12	-4.62	-7.52
RACP	Rachis hair position	-8.51	5.80	-4.04
SPCM	Spike color at maturation	-1.34	1.25	2.08
Constant		-295.00	-238.41	-300.96

TABLE 1.4. Means, standard deviations, and ranges of the variables used in the discriminant analysis of T. turgidum ssp. carthlicum

Variables	Group A	Group B	Group C
Stem height	139.6 ± 12.5	125.6 ± 12.5	139.7 ± 10.3
STHT (cm)	110 - 167	98 - 150	119 - 158
Spike density SPKD (Index)	19.5 ± 1.3	16.6 ± 1.9	18.0 ± 1.7
	16.4 - 22.0	12.8 - 22.6	15.7 - 21.4
Glume awn length - spike base GLPB (cm)	1.26 ± 0.6	1.56 ± 0.6	1.34 ± 0.6
	0.5 - 3.0	1.0 - 3.5	0.7 - 2.7
Stem solidness - middle of 2nd internode STMD (Index)	1.9 ± 0.5	2.9 ± 0.5	2.3 ± 0.4
	1.0 - 3.0	1.6 - 3.8	1.5 - 2.8
Second leaf width SLLW (cm)	1.55 ± 0.11	1.41 ± 0.11	1.66 ± 0.08
	1.3 - 1.8	1.2 - 1.7	1.5 - 1.8
Rachis hair position	1.09 ± 0.30	2.0 ± 0.0	1.16 ± 0.38
RACP	1 - 2	2	1 - 2
Spike color at maturation	1.9 ± 0.6	2.7 ± 0.4	5.0 ± 0.0
SPCM	1 - 3	2 - 3	5

TABLE 1.5. Variables selected by the stepwise discriminant analysis and classification functions for the HEXA groups.

Acronym	Description of variable	Classification functions Group 1	Group 2	Group 3
GLPT	Glume awn length - spike top	32.41	19.37	22.60
SPKD	Spike density	10.04	9.65	14.17
LOHD	Hair density -- abaxial surface	2.04	1.19	1.56
NONO	Number of internodes	47.67	33.57	33.95
INTL	First internode length	8.69	6.55	7.68
KLTP	Keel teeth position	-31.52	-18.66	-26.30
Constant		-544.80	-316.38	-452.27

TABLE 1.6. Ranges, means and standard deviations of the variables used in the discriminant analysis of the HEXA groups.

Variables	Group 1	Group 2	Group 3
Hair density abaxial surface LOHD	30.0 ± 8.7	7.4 ± 11.8	12.7 ± 10.4
	14.3 - 49.1	0.0 - 23.1	1.9 - 25.8
Spike density SPKD (Index)	14.4 ± 1.0	13.8 ± 0.56	18.3 ± 2.1
	12.1 - 16.4	13.3 - 14.6	16.7 - 21.4
Glume awn length - spike top GLPT (cm)	4.3 ± 0.69	1.6 ± 0.8	2.4 ± 0.7
	3.0 - 5.3	0.9 - 2.0	1.5 - 2.6
First internode length INTL (cm)	64.1 ± 3.8	52.7 ± 4.6	59.4 ± 3.6
	58.0 - 72.5	47.0 - 57.5	55.2 - 63.7
Number of internodes NONO	4.7 ± 0.6	4.5 ± 0.5	4.0 ± 0.0
	4 - 6	4 - 5	4
Keel teeth portion KLTP	1.1 ± 2.2	2.1 ± 1.0	1.2 ± 0.6
	1 - 2	1 - 3	1 - 2

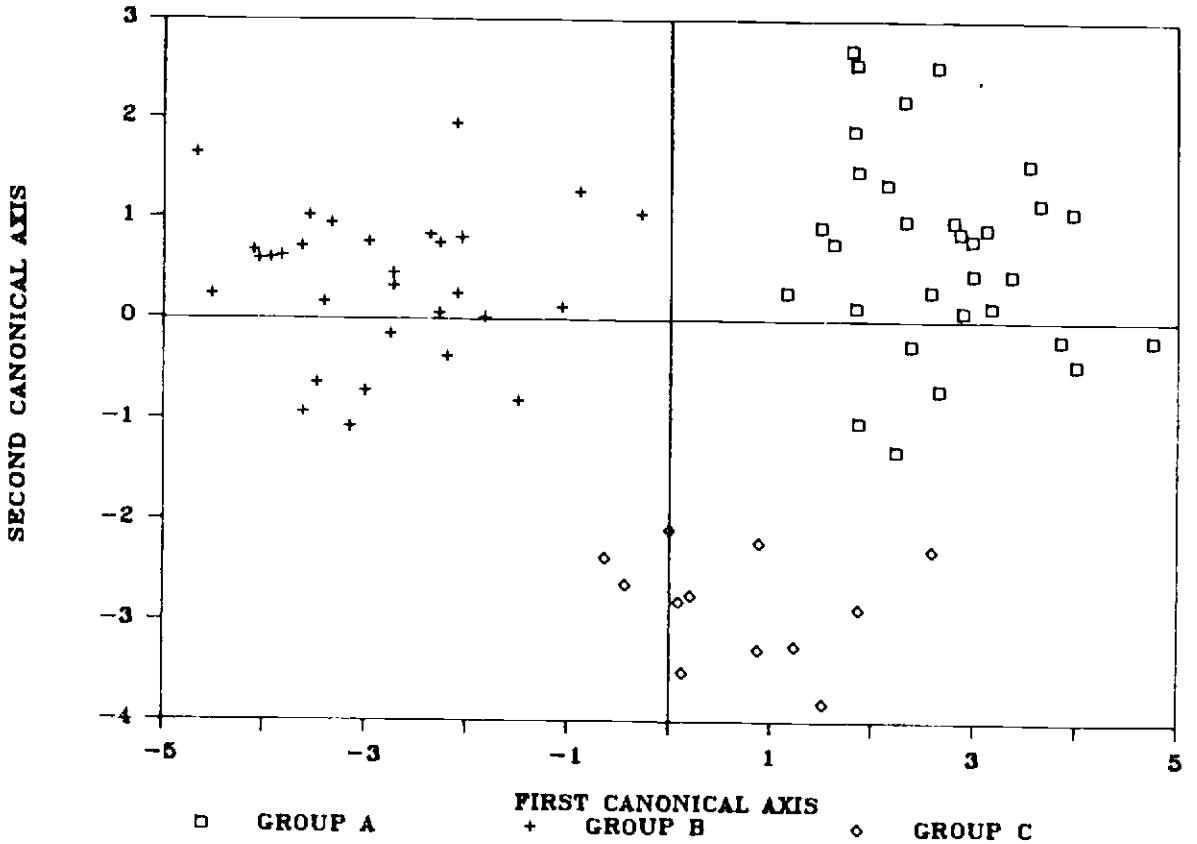

Figure 1.1. Ordination of OTU's in Tetra groups by scores defined by canonical variates 1 and 2 in discriminant analysis.

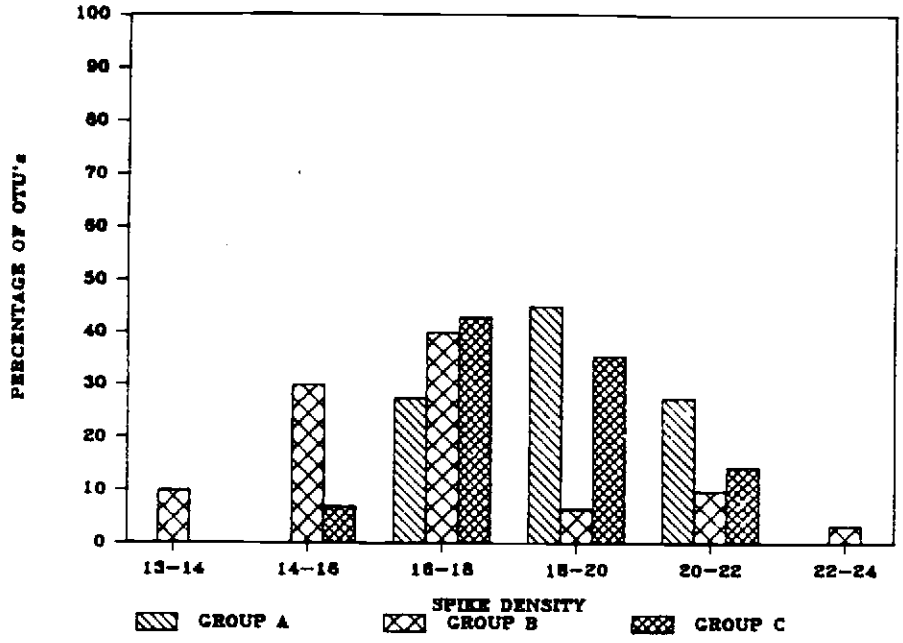

Figure 1.2. Frequency distribution of spike density (index) for groups A, B and C of T. turgidum ssp. carthlicum.

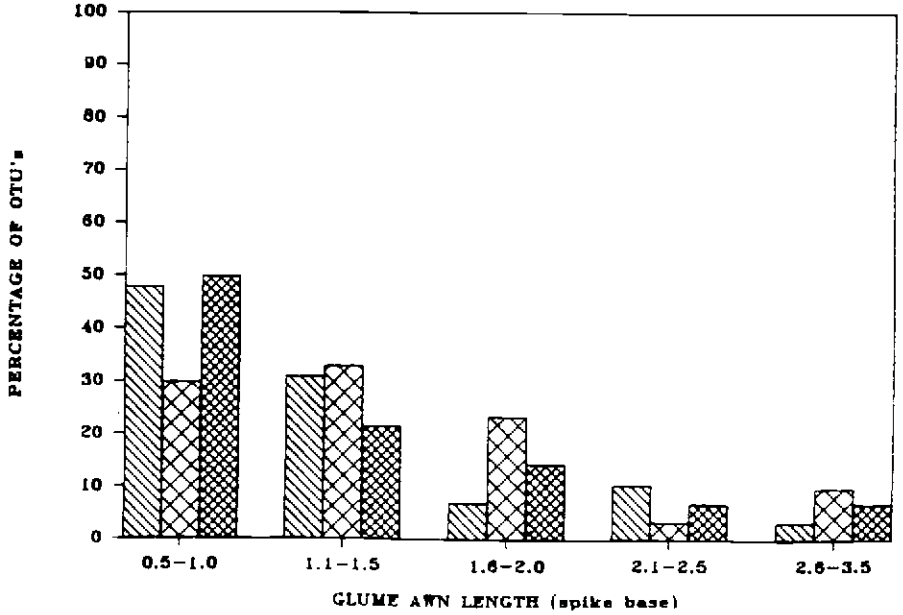

Figure 1.3. Frequency distribution of glume awn length (cm) measured at the base of the spike for groups A, B and C of T. turgidum ssp. carthlicum.

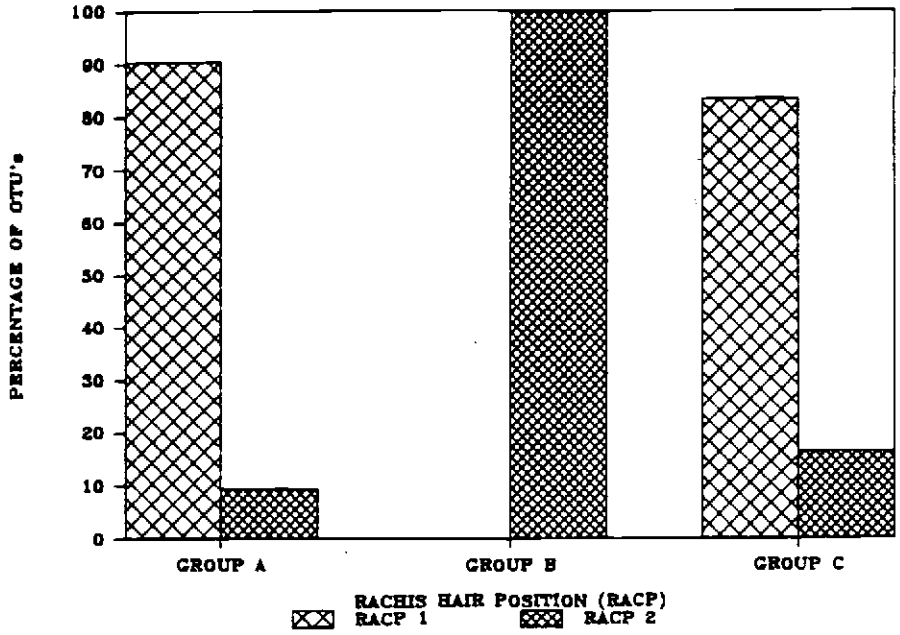

Figure 1.4. Frequency distribution of rachis hair position for groups A, B and C. of T. turgidum ssp. carthlicum.

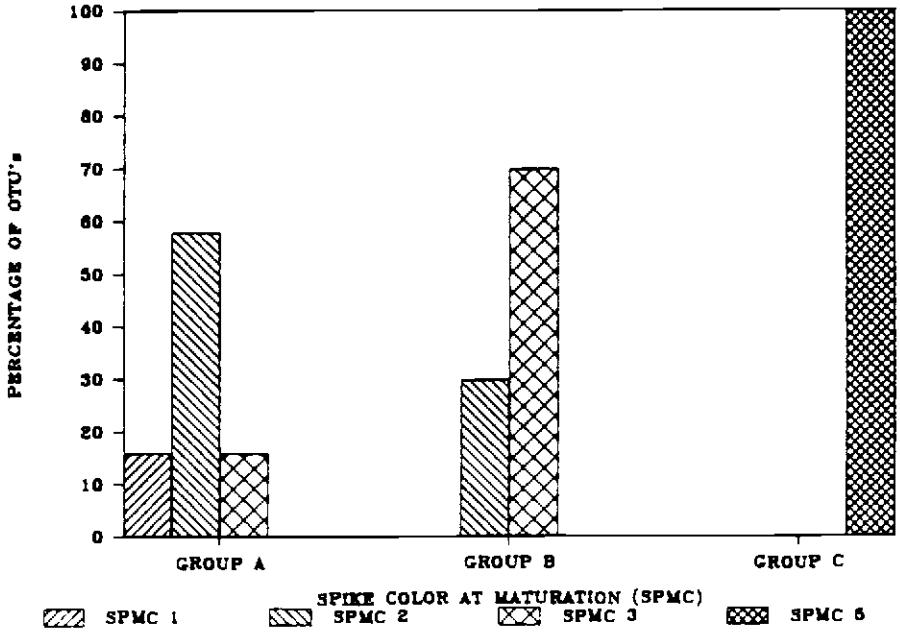

Figure 1.5. Frequency distribution of spike color at maturation for groups A, B and C. of T. turgidum ssp. carthlicum.

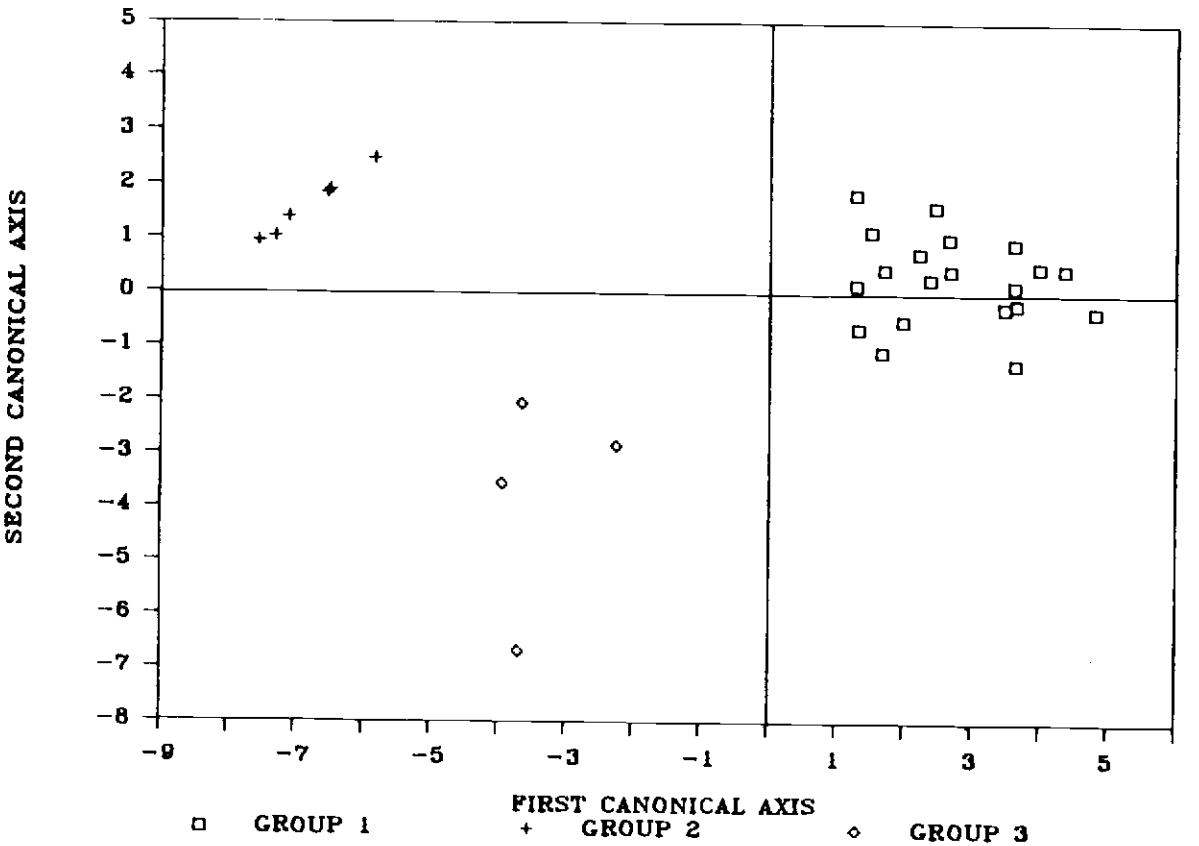

Figure 1.6. Ordination of OTU's in HEXA groups by scores defined by canonical variates 1 and 2 in discriminant analysis.

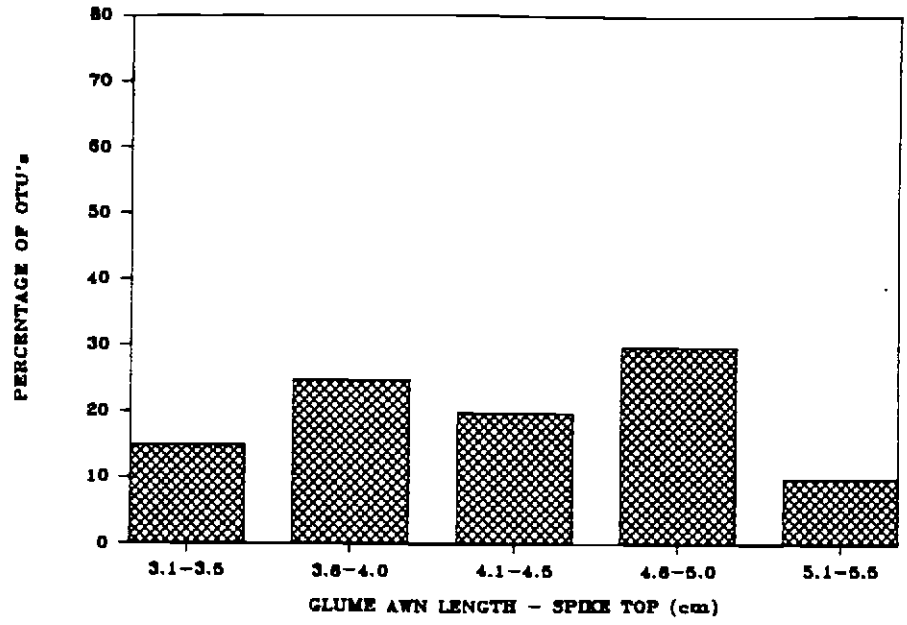

Figure 1.7. Histogram of glume awn length-spike top for group 1 (ssp. carthlicoides).

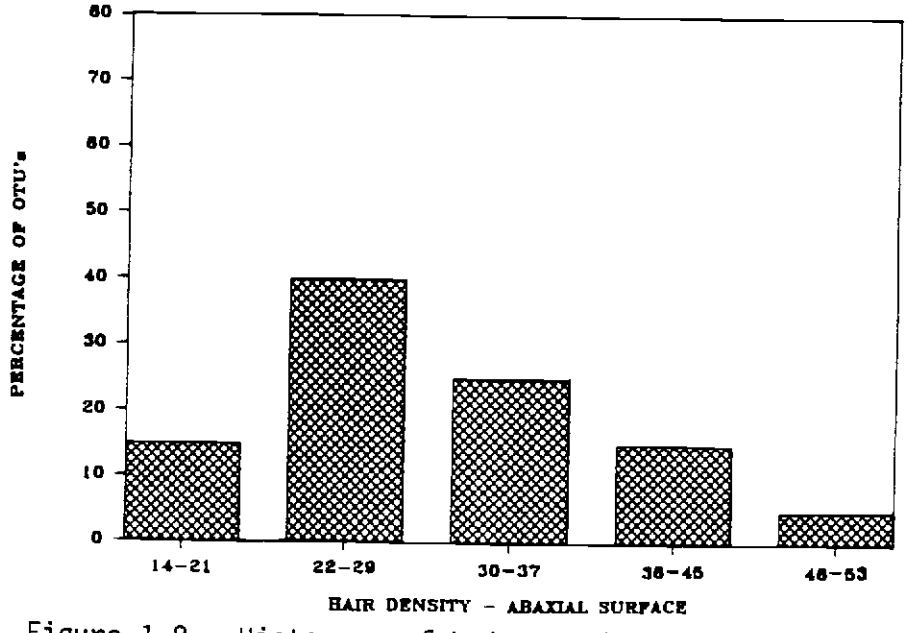

Figure 1.8. Histogram of hair density measured on the leaf abaxial surface for group 1 (ssp. carthlicoides).

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MANUSCRIPT 2

THE ORIGIN AND EVOLUTION OF *T. TURGIDUM* SSP. *CARTHLICUM* AS INDICATED BY SDS-PAGE OF HIGH-MOLECULAR-WEIGHT GLUTENIN SUBUNITS

THE ORIGIN AND EVOLUTION OF T. TURGIDUM SSP. CARTHLICUM AS INDICATED BY SDS-PAGE OF HIGH-MOLECULAR-WEIGHT GLUTENIN SUBUNITS

ABSTRACT

The phylogenetic relationship between T. turgidum ssp. carthlicum and other T. aestivum subspecies was examined based on SDS-PAGE analysis of the HMW glutenin subunits. Accessions of T. turgidum ssp. carthlicum (2n = 28 = AABB), T. aestivum ssp. carthlicoides and ssp. vulgare (2n = 42 = AABBDD), and extracted AABB tetraploid from ssp. vulgare cultivars were compared. The 73 carthlicum accessions gave identical HMW glutenin subunits profiles, indicating a monophyletic origin of this subspecies. The lack of variability also suggests its recent origin. This interpretation seems to favor the hypothesis that carthlicum is a young form derived form a cross between a Q-bearing hexaploid and a tetraploid wheat. To identify the possible hexaploid donor of the Q-factor, HMW glutenin subunits of ssp. carthlicoides and ssp. vulgare accessions were examined. The similarity in the profile presented by these two hexaploid forms indicated that either form could have been the hexaploid parent in the cross that led to the formation of carthlicum. The removal of the D genome from vulgare cultivars (represented by the extracted AABB tetraploids), however, resulted in an HMW glutenin profile closely resembles that of ssp. carthlicum, but substantially different from that of the original hexaploid cultivars. These results confirm the close relationship which appears to exist between aestivum and carthlicum. Also, the

influence of the D genome on the differential expression of HMW glutenin subunits demonstrates the limitations of interpreting evolutionary events based on the concept of simple additive relationship between genomes and protein subunits.

KEY WORDS: T. turgidum ssp. carthlicum; T. aestivum ssp. carthlicoides; wheat evolution; gel electrophoresis; glutenins.

INTRODUCTION

Triticum turgidum (L.) Thell. ssp. carthlicum (Nevski) Love et Love (2n = 28 = AABB) has been suggested as a possible progenitor of the free-threshing hexaploid wheats (Sears, 1956; Mac Key, 1966), since it is the only tetraploid form in the genus Triticum containing the Q-factor. This supposition is also supported by the resemblance of carthlicum and certain forms of T. aestivum (L.) Thell. (ssp. "carthlicoides") in spike morphology and kernel characteristics (Vavilov, 1960; Kuckuck, 1979). The observation that carthlicum is often found in mixed populations with T. aestivum ssp. carthlicoides has also lent support to the role of carthlicum in the wheat phylogeny (MacFadden and Sears, 1946; Sears, 1956; Mac Key, 1966; Kerber and Rowland, 1974; Kuckuck, 1979).

According to Vavilov (1960), the geographic distribution of ssp. carthlicum and ssp. carthlicoides is restricted to northeastern Turkey, and the Soviet Socialist Republics of Dagestan, Georgia and Armenia. The possible roles of these two forms in the evolution of wheat was reviewed by Mac Key (1966). The first hypothesis states carthlicum is the parent donor of the Q-factor in the cross with Ae. squarrosa to form the first free-threshing hexaploid. The second possibility proposes that a hexaploid with q was first formed and a subsequent cross with carthlicum gave rise to a free-threshing hexaploid. The third alternative proposes carthlicum should be regarded as the most recent form originating from the cross between a Q-bearing hexaploid and an unknown tetraploid species. Although preferences for one hypothesis or the others have been advanced

(Vavilov, 1926; Mac Key, 1954; Kihara et al., 1965; Kuckuck, 1979), no consensus has yet emerged regarding the origin of ssp. carthlicum and ssp. carthlicoides. If one accepts the first or second alternatives, ssp. carthlicoides arose as a result of the crosses. The third hypothesis states that ssp. carthlicoides was one of the parents of the cross that gave rise to ssp. carthlicum.

Electrophoretic analysis of proteins has added considerable resolution to the study of genetic diversity and phylogenetic relationship (Hall and Johnson, 1962; Johnson, 1975; Ladizinski and Hymowitz, 1979). Albumins and gliadins (two classes of storage proteins) have been analyzed in carthlicum (Johnson, 1967; Bushuk and Kerber, 1978). In T. aestivum, SDS-PAGE was used to localize genes controlling HMW glutenin subunits (Bietz et al. 1975, Lawrence and Shepherd, 1980; Payne et al. 1984a; Galili and Feldman, 1983). Available results indicate that HMW glutenin genes are located near the centromere on the long arms of chromosomes 1A, 1B, and 1D, and recombination between genes located on the same chromosome is rare (Payne et al. 1982, 1984b). Such findings are significant since genes closely linked to the centromere are less likely to undergo genetic exchange during speciation; therefore, subunits profiles of HMW glutenins should be a useful tool for the study of evolutionary events in wheat.

The primary objective of the present study was to assess the phylogenetic relationship of ssp. carthlicum based on the HMW glutenin profiles. Accessions of T. turgidum ssp. carthlicum, T. aestivum ssp. vulgare and ssp. carthlicoides were analyzed. In

addition, the cultivars Thatcher, Prelude, Rescue, and their extracted AABB tetraploids were included with the objective of examining the influence of the D genome on the expression of HMW glutenin subunits.

MATERIALS AND METHODS

Plant Material

The accessions used in this experiment were divided into the following groups: a) 74 specimens of T. turgidum ssp. carthlicum; b) 20 specimens of T. aestivum ssp. carthlicoides and 10 of ssp. vulgare; c) the commercial hexaploid varieties Thatcher, Prelude, and Rescue, and their extracted AABB tetraploids. The variety Chinese Spring was used as control in all gels.

The specimens composing the first two groups represent the entire carthlicum entries of the Small Grain Collection of U.S. Department of Agriculture and specimens collected across north-eastern Turkey, in 1979, by R.J. Metzger, J.A. Hoffmann, H.A. Sencer, and M. Kanbertay (TK collection). Detailed description of all specimens, their origin, and collection sites are published elsewhere (Vieira, 1985).

Samples of the cultivars included in the third group were kindly provided by Dr. E.R. Kerber (Department of Plant Science, University of Manitoba, Winnipeg, Canada). Seed reserves of all accessions are maintained at Oregon State University.

SDS-PAGE

The procedure used in this study is adapted from the method of Laemmli (1970), with modifications suggested by Fullington et al. (1980), Hames (1981), and Payne et al. (1981).

Wheat grains were crushed using pliers and further ground using a pestle and a mortar, and then transferred to small vials.

Proteins were extracted with 0.062 M Tris-HCl buffer (pH 6.8) containing the following: 2% (W/V) SDS, 5% (V/V) 2-mercaptoethanol, 0.001% (W/V) Bromophenol blue, 10% (V/V) glycerol. The volume of buffer used was 330 ul/10 mg flour. The samples were left at room temperature for 1 h with occasional stirring, then placed in hot water bath (95-100 C) for 3 min and stored at 4 C until used.

The Bio-Rad dual slab system was used for SDS-PAGE. Electrophoresis was carried out in vertical slab gels (160 x 140 x 1.5 mm) with 15 wells. The separating gel contained 10% (W/V) acrylamide, 0.13% (W/V) bis-acrylamide, 0.375 M Tris-HCl (pH 8.8), 0.1% SDS (W/V). The stacking gel contained 3.75% (W/V) acrylamide, 0.1% (W/V) bis-acrylamide, 0.1% (W/V) SDS, and 0.125 M Tris-HCl (pH 6.8). Both gels were polymerized with TEMED and ammonium persulfate. Each well was loaded with 25 ul of sample. A constant current of 7.5 mA was applied for about 17-18 h.

Gels were stained for 2-3 days with a staining solution of 0.02% Coomassie blue, 6% trichloroacetic acid (TCA) and 5% ethanol (Lawrence and Payne, 1983), and destained with 10% TCA (250 ml/gel). Three separated SDS-PAGE analysis were performed for each sample.

RESULTS

HMW glutenin subunit profile of T. turgidum ssp. carthlicum

The profiles of the HMW glutenin fraction of the seed protein of carthlicum lines and controls (Chinese Spring and T. aestivum ssp. vulgare, H83-1514) are presented in Figures 2.1a and 2.1b. All carthlicum accessions have identical subunits although the plant materials represent three main taxonomic groups of the subspecies (Vieira, 1985). Four bands (6, 7, 11, and 14) appear to be characteristic of carthlicum. Bands 6 and 11, also, are present in the hexaploid wheat cultivar, Chinese Spring, and are known to be controlled by genes located on the long arm of chromosome 1B (Bietz et al. 1975).

HMW glutenin profiles of T. aestivum ssp. vulgare and ssp. carthlicoides

Fourteen different bands were detected in the HMW glutenin fractions of these two subspecies (Fig. 2.2a and 2.2b). With one exception (H83-1514), all accessions had four bands in their profile. Band 9 was found only in ssp. carthlicoides, and bands 3, 5, 10, 12, and 16 were detected in ssp. vulgare only. In general, the variation within 20 specimens of ssp. carthlicoides was less than that presented among 10 accessions of ssp. vulgare. These HMW glutenin subunits were assigned to the three chromosomal groups (1A, 1B, and 1D) according to information reported by Lawrence and Shepherd (1980), Payne and Lawrence (1983), and Galili and Feldman (1983) (Fig. 2.3).

Subunits controlled by chromosome 1A are identical between the two forms. Only three accessions of ssp. carthlicoides exhibited the band combination a (band 2) (Fig. 2.3). Seventeen accessions exhibited the combination b, the null phenotype reported by Payne et al. (1980). The ssp. vulgare showed almost identical frequency for these same bands.

Greatest variation in band numbers was associated with genes located on chromosome 1B, which supports previous results (Payne et al. 1981; Galili and Feldman, 1983). Three band combinations were detected in ssp. carthlicoides, with combination c (bands 6 and 11) occurring most often.

In all cases, the pattern of bands controlled by chromosome 1D consisted of one slow- and one fast-moving band. The combination i (bands 4 and 13) was observed twice as frequent as the combination h (bands 1 and 15) in ssp. carthlicoides. In contrast, combination h was observed more frequently than the i combination in ssp. vulgare.

Comparison of the HMW glutenin profiles of ssp. carthlicum with those of T. aestivum ssp. vulgare and their AABB tetraploids.

In order to examine the influence of the D genome on the subunit pattern of HMW glutenins, tetraploids extracted from ssp. vulgare cultivars Rescue, Thatcher, and Prelude were studied. The SDS-PAGE profiles were compared with that of their parents and ssp. carthlicum (Fig. 2.4a, 2.4b).

Comparison between hexaploid Rescue and its tetraploid derivative showed that bands 4 and 13 (controlled by genes on D genome) were absent in the tetraploid as expected. However, new bands were

observed in tetraploids of cultivars Prelude and Thatcher. Bands 6, 7, and 14 of TetraPrelude were not detected in its hexaploid parent. Band 8, coded by genes on chromosome 1B , and bands 1 and 15, coded by genes on chromosome 1D, were absent following the removal of D genome. Similarly, the HMW glutenin profile on TetraThatcher showed a number of major differences when compared with its hexaploid parent. Four bands (6, 7, 11, and 14), not present in Thatcher, were detected in its derivative tetraploid. On the other hand, bands 4, 8, 12, and 13 observed in Thatcher were found to be absent from the profile of TetraThatcher. The occurrence of new HMW glutenin subunits in tetraploids appear to suggest a regulatory function of the D genome in the expression of subunits controlled by genes on homoelogous chromosomes. The removal of D genome also gave banding combinations similar to that of ssp. carthlicum (lanes F and G and compared with lane A) with the exception of the presence of band 2, which is under the control of genes on chromosome 1A. These results may indicate that extracted AABB tetraploids and ssp. carthlicum are possibly quite similar, with differences mainly residing in the A genome.

DISCUSSION

One of the major findings of the present study is the uniformity of HMW glutenin subunits of T. turgidum ssp. carthlicum. This remarkable similarity suggests a monophyletic origin of this form. As the number of accessions (74) include three taxonomic groupings of this subspecies (Vieira, 1985), the absence of variability may be representative of the subspecies as a whole. These results indicate that genomes A and B of ssp. carthlicum have not undergone substantial divergence regarding the HMW glutenin subunits composition since the origin of this allopolyploid.

The evolutionary relationships between ssp. carthlicum and ssp. T. aestivum have been speculated mainly along two approaches: carthlicum being a recent derivative originating from a cross between a Q-bearing hexaploid and a tetraploid (Vavilov, 1926; Kuckuck, 1979); alternatively, carthlicum is the original donor of the Q-factor of hexaploid wheat via a cross with Ae. squarrosa or with T. aestivum ssp. spelta (MacFadden and Sears, 1946; Mac Key, 1954). The lack of variability in HMW glutenin subunits of ssp. carthlicum as compared with the highly variable subunit patterns of the hexaploid wheats appears to favor the first alternative regarding the pathways that led to the origin of ssp. carthlicum. It is generally accepted that putative progenitor species are more genetically variable than their derivative taxon, and the level of polymorphism is associated with the length of time elapsed since the establishment of a new species (Ladizinski and Johnson, 1972; Gottlieb, 1977; Soltis, 1981).

In addition to the interpretation of ssp. carthlicum being a more recent form and, therefore, exhibiting little variation in the profile of HMW glutenin subunits, two possible alternatives must be considered. Reproductive isolation either by geographical isolation or by low hybrid fitness may explain the absence of variability. However, ssp. carthlicum is commonly found growing in mixed fields of Triticum aestivum ssp. carthlicoides species (Metzger, personal communication; Tsvelev, 1984), it crosses readily with other wheat species (Vavilov, 1925) and viable individuals are found among the progenies of these crosses (Vavilov, 1925; Mac Key, 1966). Another possibility is that the protein loci are implicated with fitness of individuals in specific environmental conditions. However, storage proteins are characterized by the absence of other known functions besides providing a source of nitrogen for the developing seedling (Pernollet and Mosse, 1983; Higgins, 1984).

The HMW glutenin profile of ssp. carthlicoides presented less variability than ssp. vulgare. However, two subunits (6 and 11) always present in the carthlicum profile were equally observed in some accessions of both hexaploid subspecies. These results indicated that, based on HMW glutenin profile alone, either form could be considered has having participated in the formation of ssp. carthlicum. Also, one cannot exclude the possibility that ssp. carthlicoides is a hexaploid derivative from the same cross (ssp. vulgare x tetraploid wheat) that also gave rise to ssp. carthlicum.

Our analysis of the HMW glutenin profiles in hexaploid wheats and their derived AABB tetraploids showed that intergenomic gene

interactions can have dramatic qualitative effects on the expression of seed storage proteins. As expected, the removal of the D genome from the hexaploid cultivar Rescue resulted in the deletion of bands coded by genes located on chromosome 1D. On the other hand, the profiles of cultivars Thatcher and Prelude were greatly altered with the extraction of the D genome. With exception of bands 0 and 2, coded by genes on chromosome 1A, all HMW glutenin subunits of the typical carthlicum profile were observed in TetraThatcher and TetraPrelude. These results again support the hypothesis that ssp. carthlicum and ssp. vulgare are closely related from the standpoint of history and evolution.

It has been suggested that the additive nature of seed storage proteins makes them extremely useful for evolutionary and taxonomic studies (Hall and Johnson, 1962; Murray et al., 1970; Ladizinski and Hymowitz, 1979). However, as demonstrated by the regulatory effect of the D genome on the expression of HMW glutenin subunits, the profile of a polyploid species may not represent the sum of the putative parental patterns.

Enhancement in expression of genes controlling storage proteins caused by the prevention of expression of homoegenes (Aragoncillo et al., 1978) can account for the differential expression of HMW glutenin subunits caused by the loss of the D genome. Also, changes in transcriptional controls caused by regulatory genes (Burr and Burr, 1982; Thompson and Bartels, 1983) and post-translation mechanisms (Yamagata et al., 1982) should be considered in attempts to explain the non-additive protein patterns found in this study.

Figure 2.1. SDS-PAGE of HMW glutenin subunits (1a) and diagram representing group-specific bands (1b) of ssp. carthlicum and Chinese Spring.

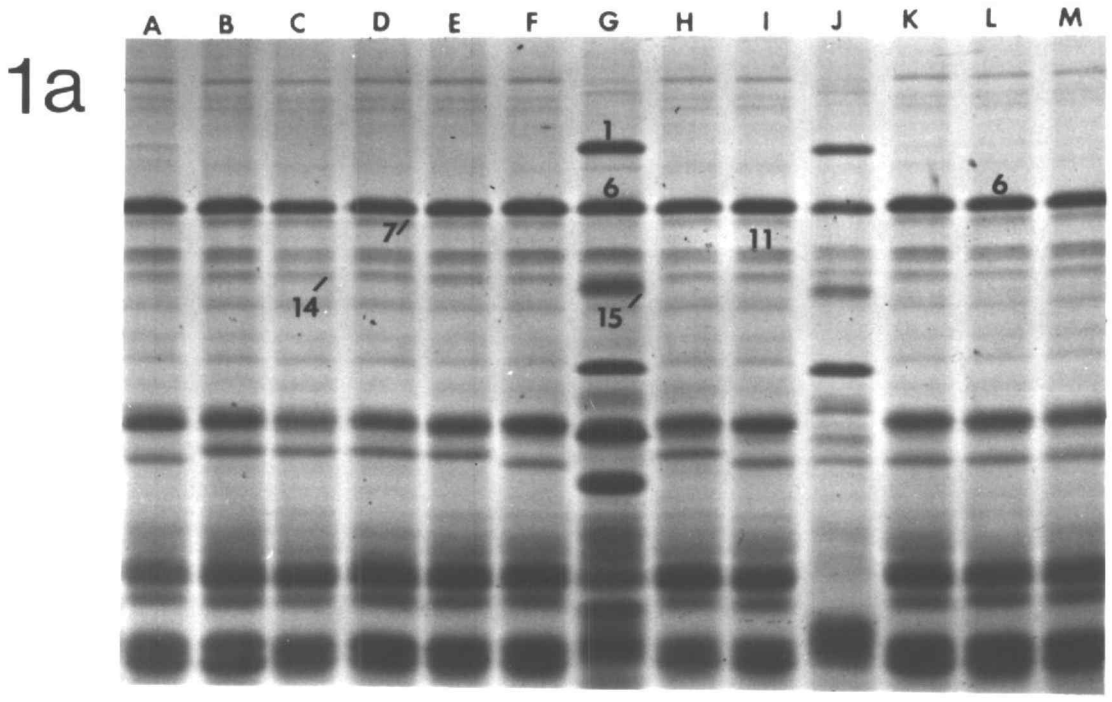
Lanes A to F: ssp. carthlicum, accessions H83-1502, H83-1503, H83-1504, H83--1505, H83-1507, H83-1509.

Lane G: Chinese Spring.

Lanes H and I: ssp. carthlicum, accessions H83-1511.

Lane J: ssp. vulgare, accessions H83-1514.

Lanes K to M: ssp. carthlicum, accessions H83-1515, H83-1517, H83-1519.


1a
A
B
C
D
E
F
G
H
I
J
K
L
M
1
6
7
14
15
11
6

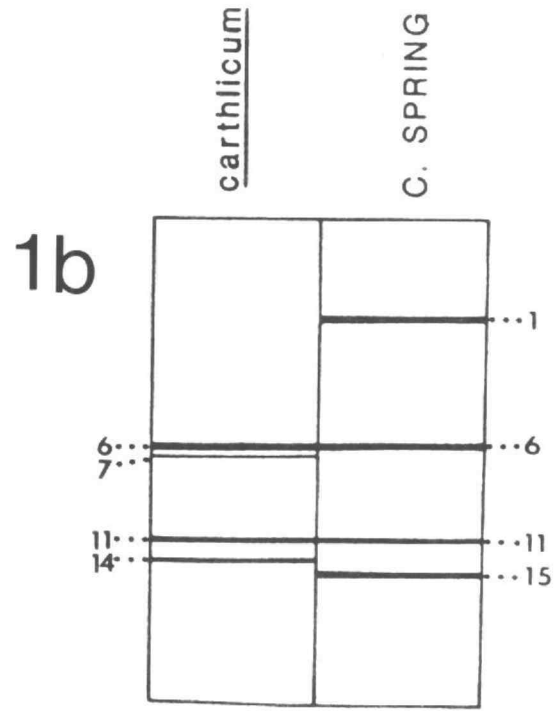
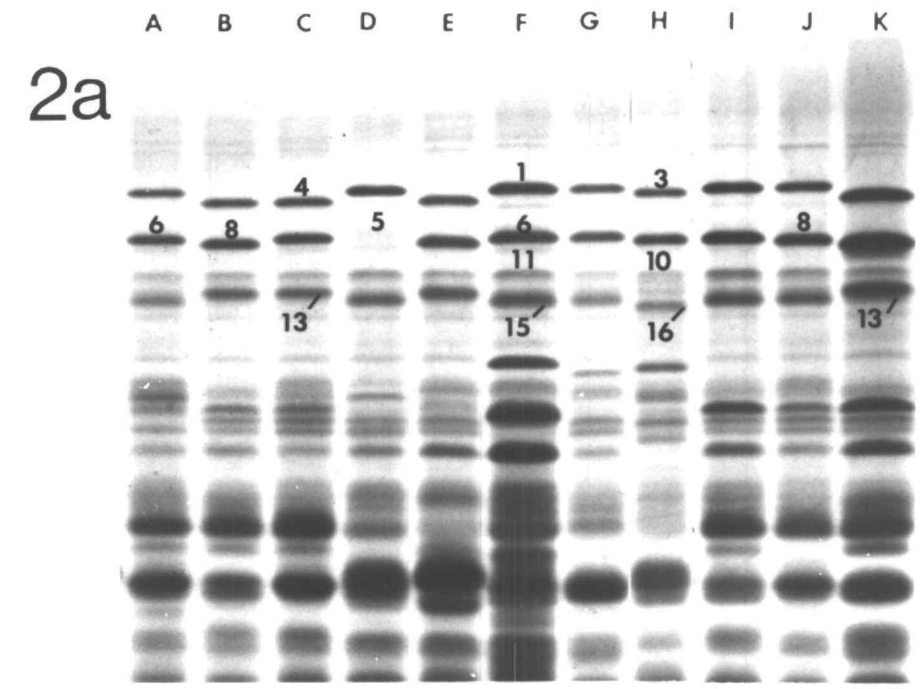

carthlicum
C. SPRING
1b
1
6
7
11
14
6
11
15

Figure 2.1

Figure 2.2. SDS-PAGE of HMW glutenin subunits of *T. aestivum* ssp. *vulgare* (2a) and ssp. *carthlicoides* (2b).

Gel 2a: (A) H83-1530; (B) H83-1531; (C) H83-1554; (D) H83-1544; (E) H83-1561; (F) Chinese Spring; (G) H83-1582; (H) H83-1514; (I) H83-1567; (J) H83-1553; (K) H83-1535.

Gel 2b: (A) H83--1529; (B) H83-1543; (C) H83-1512; (D) H83-1516; (E) H83-1518; (F) H83-1527; (G) Chinese Spring; (H) H83-1506; (I) H83-1520; (J) H83-1521; (K) H83-1523.


2a
A
B
C
D
E
F
G
H
I
J
K
6
8
4
13
5
1
6
11
15
3
10
16
8
13

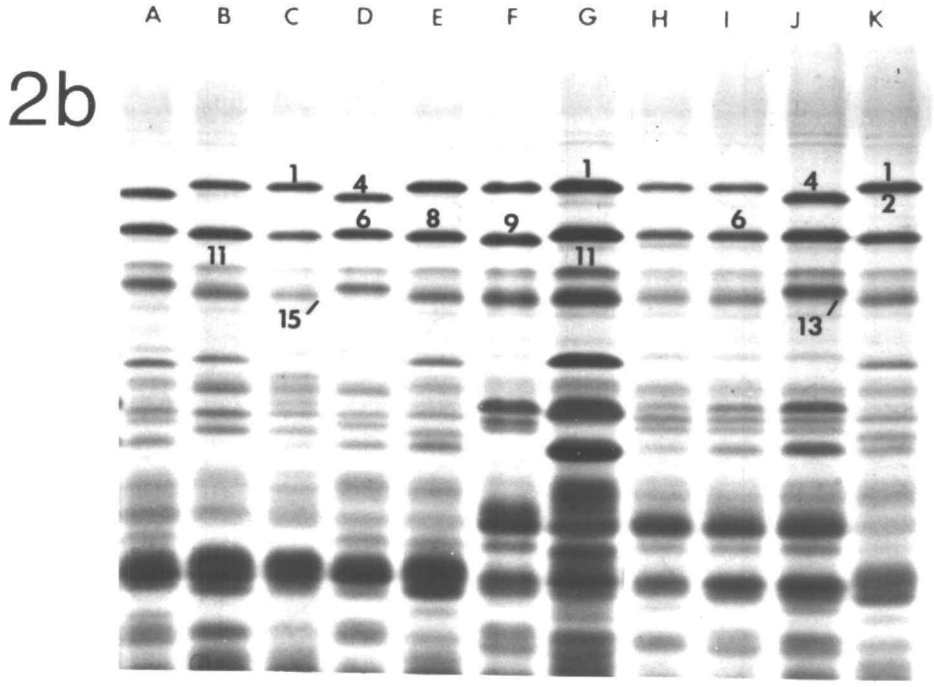

2b
A
B
C
D
E
F
G
H
I
J
K
11
1
15
4
6
8
9
1
11
6
4
13
1
2

Figure 2.2

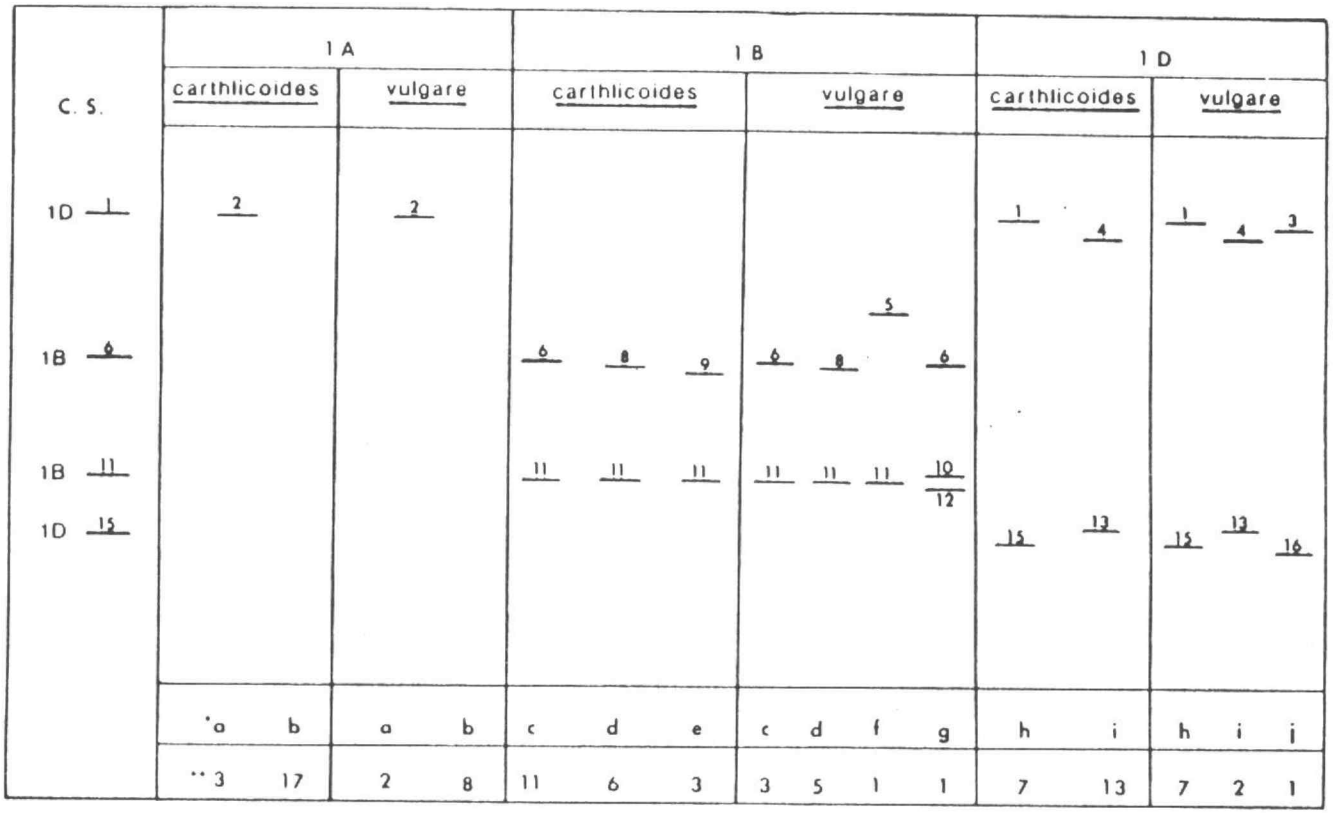


Figure 2.3. Diagram of HMW glutenin subunits of ssp. carthlicoides and ssp. vulgare assigned to group 1 homoeologous chromosomes. On the left of diagram are the standard bands from Chinese Spring.

*Combination of band pattern
**Number of accessions with that combination of band pattern

Figure 2.4 SDS-PAGE of HMW glutenin subunits (4a) and diagram representing group-specific bands (4b) of ssp. carthlicum, ssp. vulgare cultivars and extracted AABB tetraploids.

Lane A : ssp. carthlicum

Lane B : TetraRescue

Lane C : Rescue

Lane D : Chinese Spring

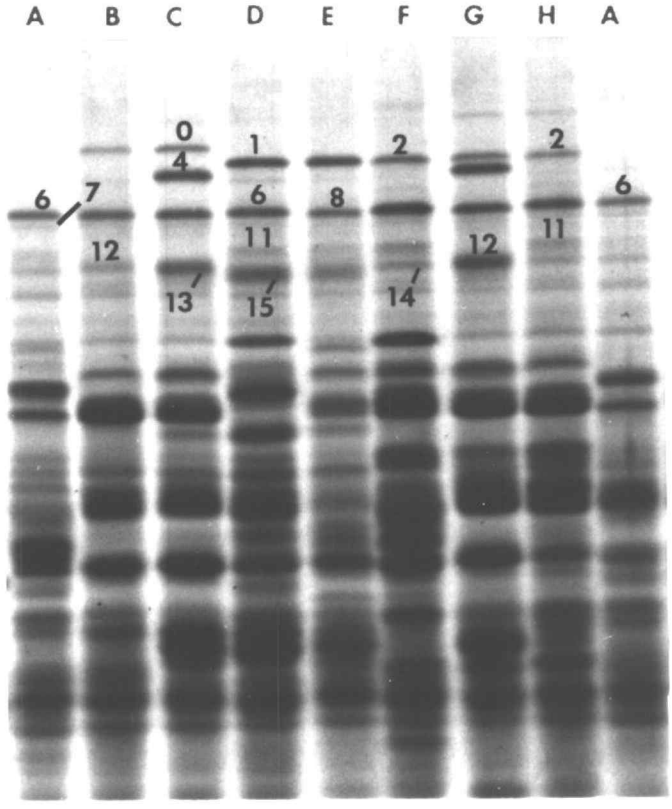
Lane E : Prelude

Lane F : TetraPrelude

Lane G : Thatcher

Lane H : TetraThatcher

4a



4b

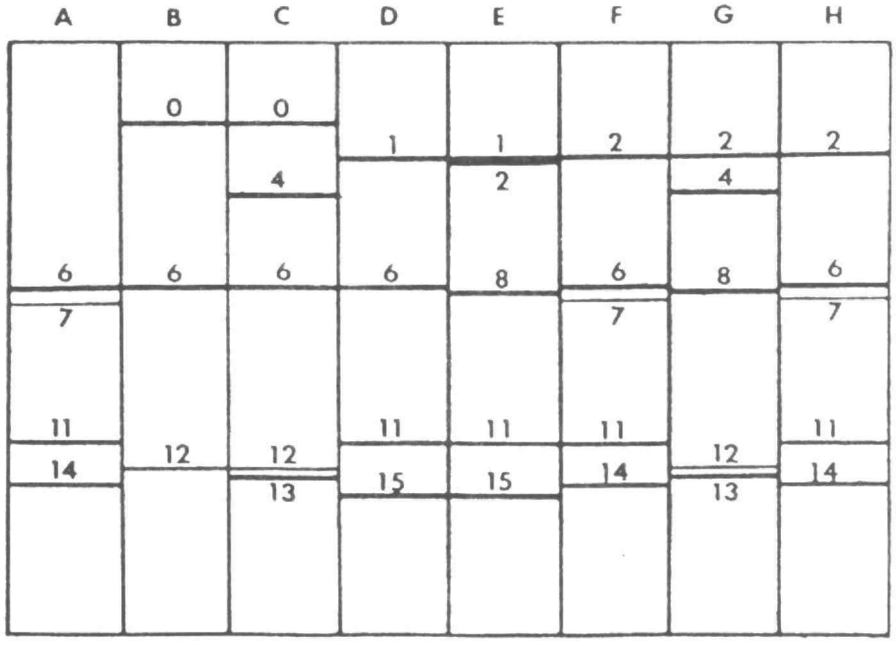


Figure 2.4

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Zhukovski, P.M. 1923. Persian wheat - Triticum persicum Vavilov in Transcaucasia. Bull. Appl. Bot. Plant Breed. 13, 45-58.

A P P E N D I X

APPENDIX TABLE 1. Collection sites of Turkey expedition in 1979, carried out by R.J. Metzger, J.A. Hoffmann, H.A. Sencer, and M. Kanbertay, which provided the material used in this investigation.

Site #	Province	Precise locality	Altitude (m)
TK97	Kars	13 kms. N.E. Sarikamis	1880
TK98	Kars	12 kms. S.W. Arpacay	1590
TK99	Kars	9 kms. N.W. Arpacay	1920
TK100	Kars	10 kms. S. Dogruyol	1900
TK101	Kars	4 kms. S.E. Cildir	1900
TK103	Kars	29 kms. W. Cildir	1800
TK104	Kars	24 kms. N.W. Kars	1700
TK107	Kars	19 kms. N. Gole	1880
TK108	Kars	40 kms. N. Gole	1840
TK110	Erzurum	33 kms. N.E. Erzurum	2010
TK112	Erzurum	18 kms. N.E. Erzurum	1810
TK123	Gumuchane	37 kms. S.E. Bayburt	1920

APPENDIX TABLE 2. *Triticum turgidum* ssp. *carthlicum* specimens assigned to group A by the numeral analysis.

O.T.U.	Collection number or P.I. / C.I. number
H83-1509	TK99-526 B-1
H83-1510	TK99-526 B-2
H83-1546	PI 94748
H83-1547	PI 94749*
H83-1654	PI 94749*
H83-1548	PI 94750
H83-1549	PI 94751
H83-1551	PI 94753
H83-1553	PI 94755
H83-1603	PI 352279
H83-1655	PI 352779
H83-1604	PI 352280
H83-1605	PI 352281
H83-1536	CI 7692
H83-1563	PI 283887
H83-1564	PI 283888
H83-1565	PI 283889
H83-1566	PI 283890
H83-1535	PI 272522*
H83-1592	PI 190949*
H83-1597	PI 190949*
H83-1569	PI 251914
H83-1600	PI 286070
H83-1601	PI 286071
H83-1595	PI 287071
H83-1579	55B4.263.1
H83-1627	CI 7692
H83-1599	PI 272522*
H83-1541	55B4.263.2
H83-1502	TK 97-500 B, hd. 2
H83-1507	TK 98-517, hd. 1

*Material with same PI or CI number were obtained from one source but, due to dissimilarity, were regarded as independent O.T.U.

APPENDIX TABLE 3. *T. turgidum* ssp. *carthlicum* specimens accessions assigned to group B by the numerical analysis.

O.T.U.	Collection or P.I. / C.I. numbers
H83-1503	TK97-503, hd. 8
H83-1504	TK97-503, hd. 18
H83-1505	TK97-503, hd. 58
H83-1617	TK97-503, hd. 13
H83-1609	TK97-503-34
H83-1651	TK97-503-34
H83-1633	TK98-510, hd. 1
H83-1610	TK98-517-5
H83-1614	TK99-522A, hd. 3
H83-1615	TK99-522A, hd. 1
H83-1640	TK99-522A
H83-1636	TK99-522B, hd. 1
H83-1532	TK100-532D-2
H83-1511	TK100-531A, hd.2
H83-1612	TK100-532D-2
H83-1515	TK103-544A
H83-1608	TK103-544A-2
H83-1517	TK104-548A, hd. 1
H83-1519	TK104-552A, hd. 1
H83-1522	TK107-732, hd. 8
H83-1533	TK108-572C
H83-1524	TK108-574, hd. 1
H83-1559	PI 115817
H83-1572	PI 349040
H83-1557	CI 10111
H83-1598	PI 272521*
H83-1537	--
H83-1538	55B4.272
H83-1581	PI 272521*
H83-1528	TK108-572-3, hd. 2

*Material with same PI or CI number were obtained from one source but, due to dissimilarity, were regarded as independent O.T.U.

APPENDIX TABLE 4. T. turgidum ssp. carthlicum accessions assigned to group C by the numerical analysis.

O.T.U.	Collection or P.I. / C.I. numbers
H83-1550	94752
H83-1552	94754
H83-1558	115816
H83-1580	341800*
H83-1571	341800*
H83-1573	349041
H83-1602	352278
H83-1606	352282
H83-1556	10110*
H83-1656	10110*
H83-1534	55B4.263
H83-1578	55B4-260

*Material with same PI or CI number were obtained from one source but, due to dissimilarity, were regarded as independent O.T.U.

APPENDIX TABLE 5. Jackknifed classification matrix for tetra groups.

Group	Number of cases classified as A	B	C	% correct
A	28	0	0	100
B	0	29	0	100
C	3	1	12	75

APPENDIX TABLE 6. Groupings of *T. aestivum* accessions according to the numerical analysis.

O.T.U.	Collection or P.I. / C.I. numbers
GROUP 1	
H83-1501	TK97-500, hd. 1
H83-1506	TK-503, hd. 1
H83-1512	TK100-531A, hd. 3
H83-1516	TK103-544B-1
H83-1518	TK104-548B, hd. 1
H83-1520	TK104-552B, hd. 1
H83-1521	TK107-732, hd. 5
H83-1523	TK108-573, hd. 1
H83-1527	TK108-572-3, hd. 1
H83-1529	TK112-1067, hd. 6
H83-1543	PI 262678
H83-1562	PI 182472
H83-1568	PI 341415
H83-1574	PI 349042
H83-1611	TK99-531A
H83-1616	TK99-522B, hd. 2
H83-1634	TK98-510, hd. 2
H83-1637	TK110-603B, hd. 2
H83-1638	TK123-645
H83-1657	PI 272522
GROUP 2	
H83-1514	TK101-540D
H83-1530	TK112-1067C
H83-1531	TK112-1068, hd. 2
H83-1544	CI 7907
H83-1582	--
H83-1653	TK101-540D
GROUP 3	
H83-1554	PI 94756
H83-1561	PI 182471
H83-1567	PI 330539
H83-1635	TK100-532D, hd. 1

APPENDIX TABLE 7. Jackknifed classification matrix for hexa groups.

Group	Number of cases classified as 1	2	3	% correct
1	20	0	0	100
2	0	6	0	100
3	0	0	4	100

APPENDIX TABLE 8. Tentative comparisons between bands nomenclature used in this study to the system proposed by Payne and Lawrence (1983) - I, and Galili and Feldman (1983) - II.

Band nomenclature in this study	I	II
0	1	A1
1	2	D1
2	2*	A2
3	4	D2
4	5	D3
5	6	B1
6	7	B2
7	-[+]	--
8	-	B3
9	-	B4
10	-	B9
11	8	B10
12	9	B12
13	10	D4
14	-	--
15	12	D5
16	-	D6

[+]Bands with no homology to the ones detected by the mentioned authors.

APPENDIX TABLE 9. Gel system.

This procedure is adapted from the method of Laemmli (1970). It also incorporates modifications based on those of Fullington et al. (1980), Hames (1981), Payne et al. (1981), and by trial and error in our laboratory.

Protein extraction

The **extraction buffer solution** is prepared as follows:

Distilled water	16.0 ml
0.5 M Tris - HCL, pH 6.8	4.0 ml
10.0% (w/v) SDS	6.4 ml
Glycerol	3.2 ml
0.05% (w/v) Bromophenol blue	0.8 ml

This amount is enough for approximately 30 samples at 1.0 ml per sample.

Crush the seeds to small pieces with pliers. The samples are further ground using a pestle and a mortar and transferred (30 mg) to 60 x 15 mm tubes.

Under the hood add 1.6 ml of 2-mercaptoethanol to the extraction buffer solution. This mixture is kept under hood until extraction procedure is completed.

The volume of buffer used per sample is adjusted to have 10 mg flour/330 ul of buffer.

Shake the tubes (VORTEX) to assure that all material is wetted and allow to stand for 1 hour at room temperature.

Incubate the sample in boiling water for 3-5 minutes. Allow to cool to room temperature.

If the sample is not used immediately, store at 4 C until electrophoresed. For long period storage, keep in the freezer at -20 C.

Acrylamide gels

(1) Stock solutions for gel making.

Gel Buffer A - 0.5 M Tris - HCL, pH 6.8

Tris (Trizma base)	60 g
Distilled water	40.0 ml

Adjust to pH 6.8 with 2N HCL
To 100 ml with distilled water

Store at 4 C. Remains stable for several weeks.

Gel Buffer B - 3.0 M Tris - HCL, pH 8.8

Tris (Trizma base)	36.3 g
Distilled water	30.0 ml

Adjust to pH 8.8 with 2N HCL
To 100 ml with distilled water

Store at 4 C. Remains stable for several weeks.

Acrylamide Stock Solution

Acrylamide	30.0 g
Bis - acrylamide	0.4 g

To 100 ml with distilled water

Store in dark bottle at 4 C. Remains stable for several months.

10% (w/v) SDS

5 g of SDS dissolved in approximately 30 ml of distilled water. Bring to 50 ml with distilled water. Store at room temperature for several weeks. High quality SDS is recommended to avoid difficulties in staining.

1.5% (w/v) Ammonium Persulfate

Dissolve 0.15 g ammonium persulfate in 10 ml distilled water. This solution must be freshly prepared every time before use.

TEMED - Stable if kept in dark bottle at 4 C.

(2) Gel Preparation

Resolving gel (10%) - Bis 0.13%

Distilled water	28.9 ml
Acrylamide stock solution	20.0 ml
3.0 M Tris - HCL, pH 8.8 (buffer B)	7.5 ml
10% (w/v) SDS	0.6 ml

TEMED	40.0 ul
1.5% (w/v) ammonium persulfate	3.0 ml

The above amounts of the various solutions are enough for making 2 gels 160 x 140 x 1.5 mm.

Stacking Gel (3.75%)

Distilled water	11.3 ml
Acrylamide stock solution	2.5 ml
0.5 M Tris - HCL, pH 6.8 (Buffer A)	5.0 ml
10% (w/v) SDS	0.2 ml
TEMED	20.0 ul
Ammonium persulfate	1.0 ml

The Bio-Rad dual slab system is used in this procedure. The glass plates are washed with detergent, rinsed with distilled water and 95% ethanol before assembling in the casting stands. Spacers of 1.5 mm are used between the glass plates.

For the resolving gel preparation mix, all the ingredients except the TEMED and ammonium persulfate. Add these immediately before pouring the gels and mix gently. The solution is delivered to the slabs with a hypodermic syringe (50 to 60 ml capacity). A space of about 4 cm is left at the top of the slabs for the stacking gel and wells.

Overlay the resolving gel solution with distilled water immediately after pouring it. This step is critical to ensure a smooth and flat gel surface. The water has to be layered at a slow and constant rate, without disturbing the gel surface. Hold the syring needle just above the gel solution, starting in the middle of the slab. Then, move along the entire gel surface, in both directions, always maintaining an even delivery rate.

Layer until about 1 cm of water has been added (approximately 1.5 ml).

The polymerization takes about 20-30 minutes as indicated by the formation of a distinct interface between the water and the gel.

The process can be stopped at this time and the gels kept for days if the evaporation of the water is prevented (seal with Parafilm or preferably store in refrigerator).

The stacking gel is poured in the slabs just before use, in the same way as the resolving gel.

Remove the overlay water and rinse with distilled water at least twice. The combs are placed between the glass plates on a angle so to avoid trapping air bubbles under the teeth. Pour the stacking gel solution almost to the top of the glass plates and then

adjust the combs. There is no need to layer with water. Allow the gel to polymerize for 20-30 minutes.

Electrophoresis

Electrophoresis Buffer (10 x stock solution)

Tris (Trizma base)	30.3 g
Glycine	144.0 g
SDS	10.0 g
To 1000 ml with distilled water	

Store at room temperature. Stable for several weeks.

Loading the samples

The combs are gently removed from the top of the gel plates. Rinse each gel with distilled water, drain and rinse with electrophoresis buffer.

Load the samples (25-30 ul) in the wells using a micropippet or syringe.

Overlay the protein samples, with electrophoresis buffer, carefully to avoid contamination from each other when the buffer is loaded onto the upper tank of the unit.

Running the gels

Dilute the 10X concentrated stock solution of the electrophoresis buffer ahead of time. For 4000 ml of the buffer - 600 ml in the upper tank and 3400 in the lower - dilute 400 ml of the 10X stock solution with 3600 ml of distilled water. This buffer is prepared fresh each time from the stock solution.

Connect the negative terminal in the upper tank and the positive in the lower tank. Put a stirring bar in the lower tank and place the electrophoresis unit on a magnetic stir plate to circulate the buffer.

For a high resolution of the HMW protein bands, the current at which the samples are run, combined with the duration of the run are very important. Good results are obtained at a constant current of 7.5 mA/gel during 17-18 hours.

Staining and Destaining

Stain stock solution

Coomassie Blue R - 250 2.0 g
To 500 ml with 95% ethanol

Filter the dye stock solution with filter paper (Whatman #1) just before use.

Dilute the stock solution in 950 ml of water containing 60 g of trichloroacetic acid. This will give the final concentrations:

0.02% Coomassie Blue
6% TCA
5% Ethanol

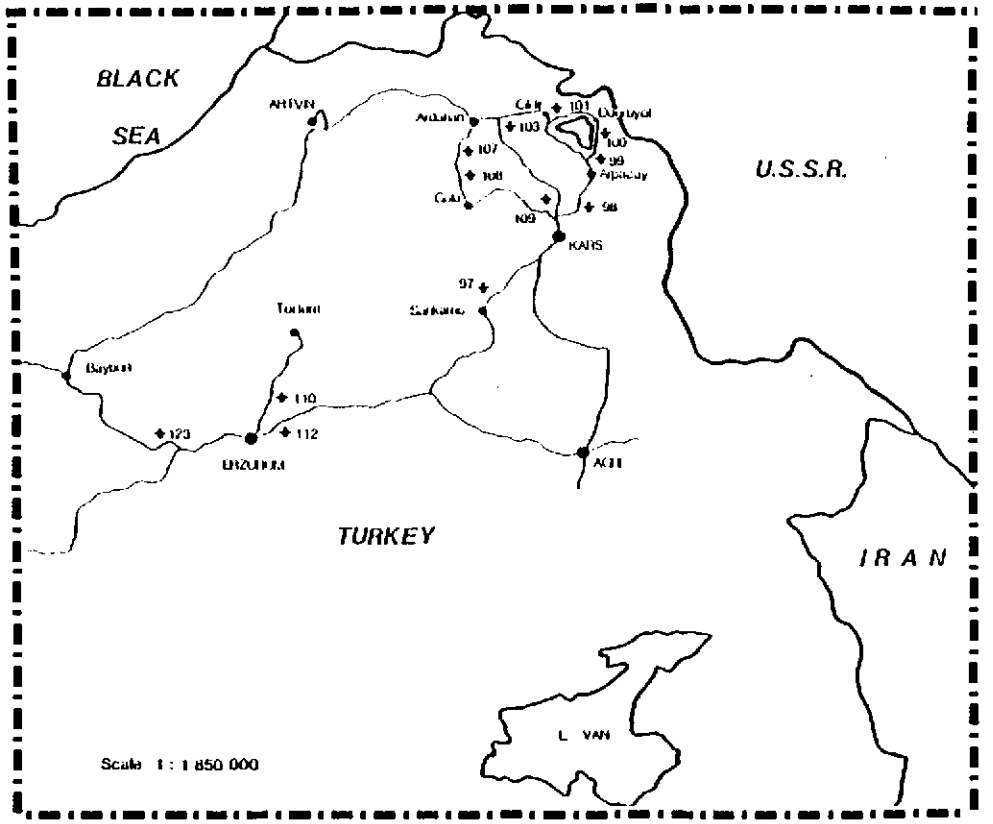
Pry the glass plates apart using a plastic object to avoid breaking the plates. Remove one of the plates and squirt distilled water between the gel and the glass plate to which the gel is adhering.

Allow the gel to fall off into a tray. Enough staining solution is added to cover the gels, usually about 500 ml/gel.

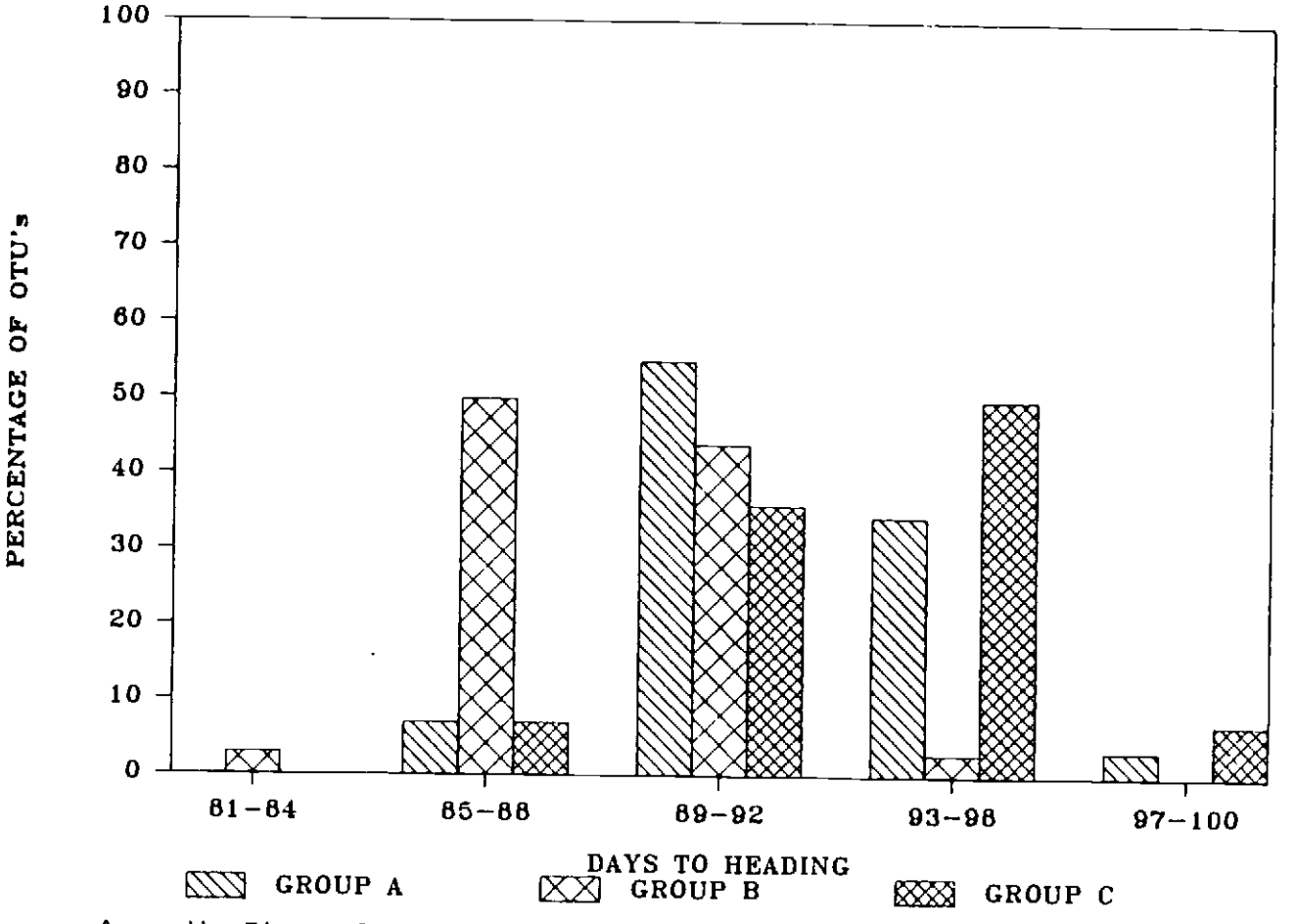
Gels are kept in the staining solution for 2-3 days before destaining. The gels are destained with 10% TCA (250 ml/gel) 1 day before taking photographs.

Gels are photographed with 35 mm camera. Panatomic X - ASA 32 film, using a yellow-orange filter.

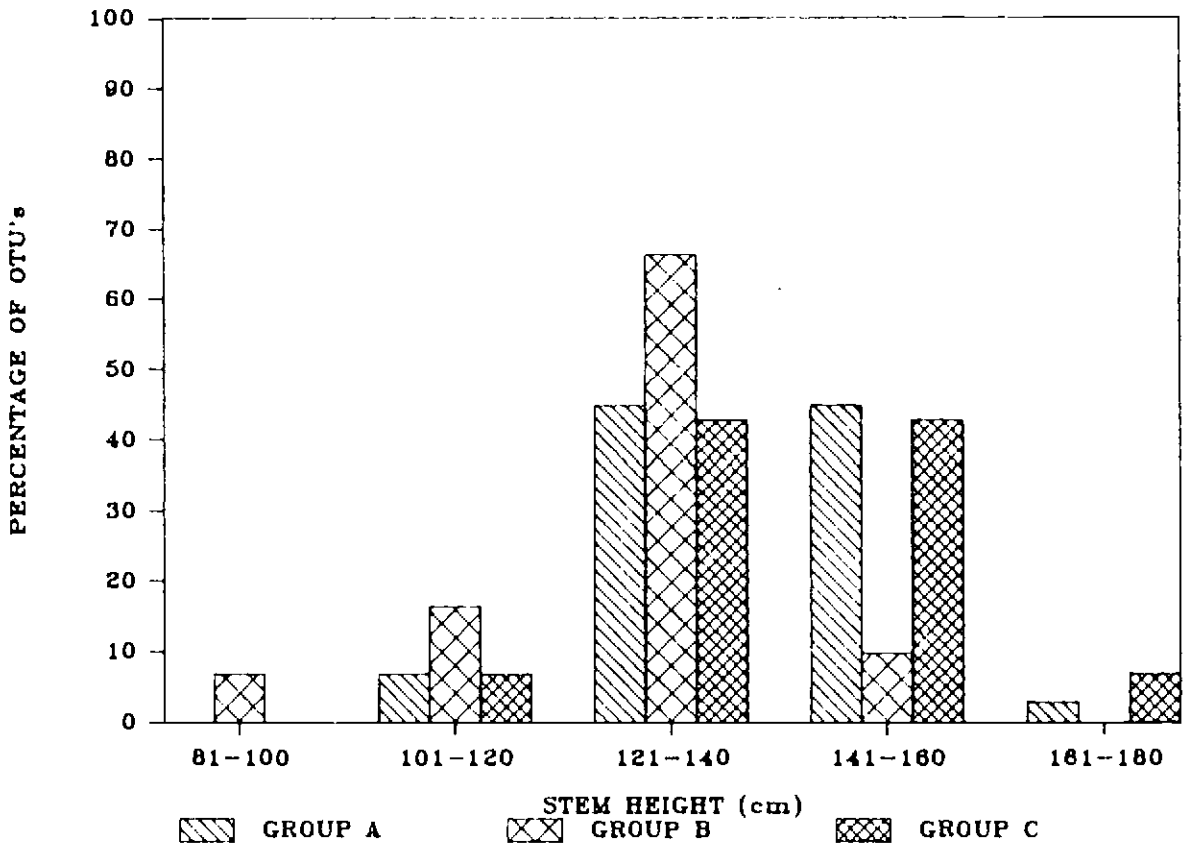
The gels can be stored in the dark in 10% TCA solution at 4 C. Prolonged storage in this condition can cause the gel to shrink.



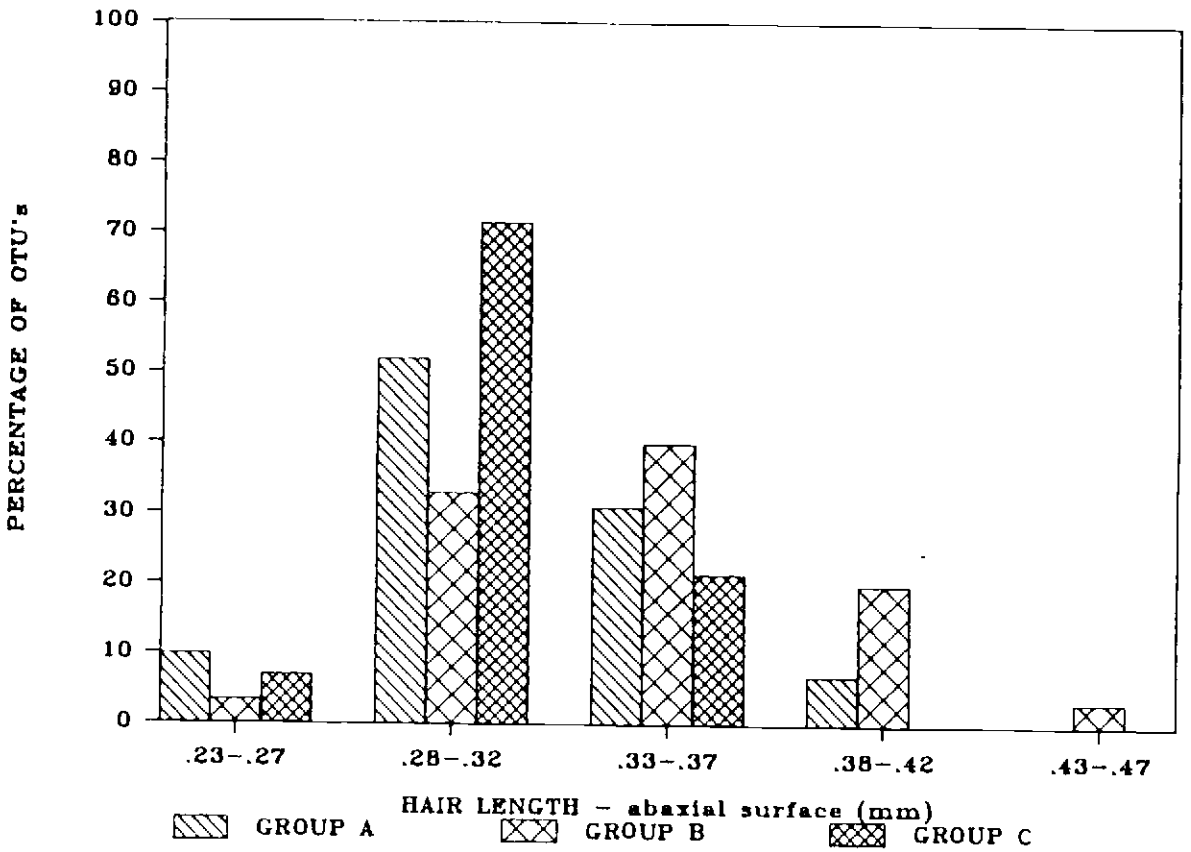
APPENDIX FIGURE 1. Route of the collection in northeastern Turkey. The collection sites of the specimens used in this investigation are also indicated.



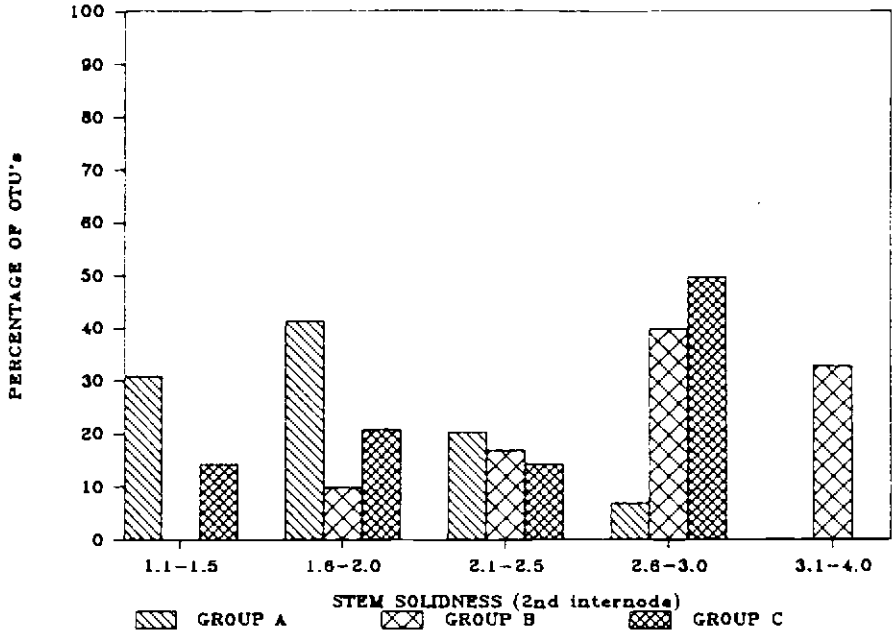
Appendix Figure 2. Frequency distribution of days to heading in groups A, B and C of T. turgidum ssp. carthlicum.



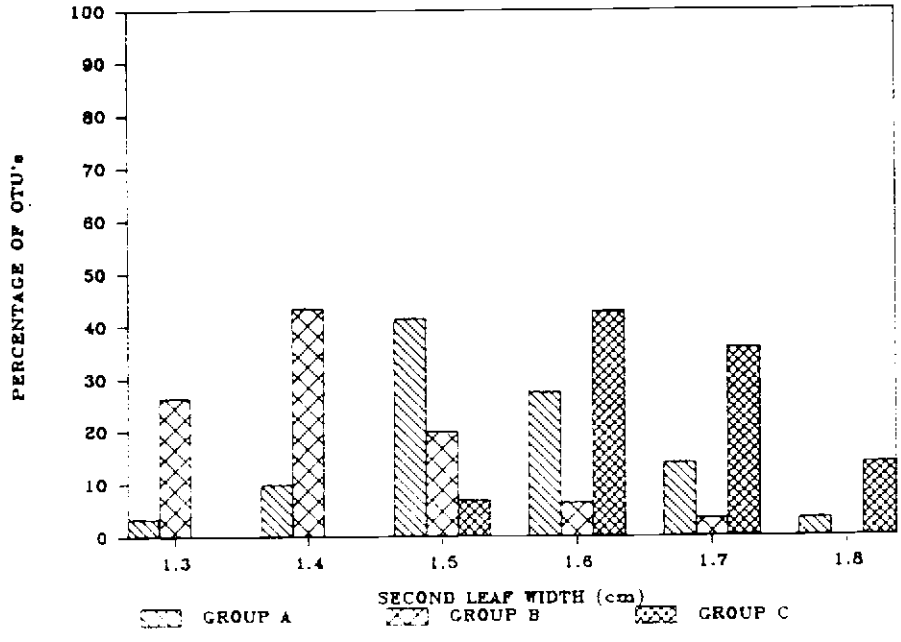
Appendix Figure 3. Frequency distribution of stem height (cm) in groups A, B and C of T. turgidum ssp. carthlicum.



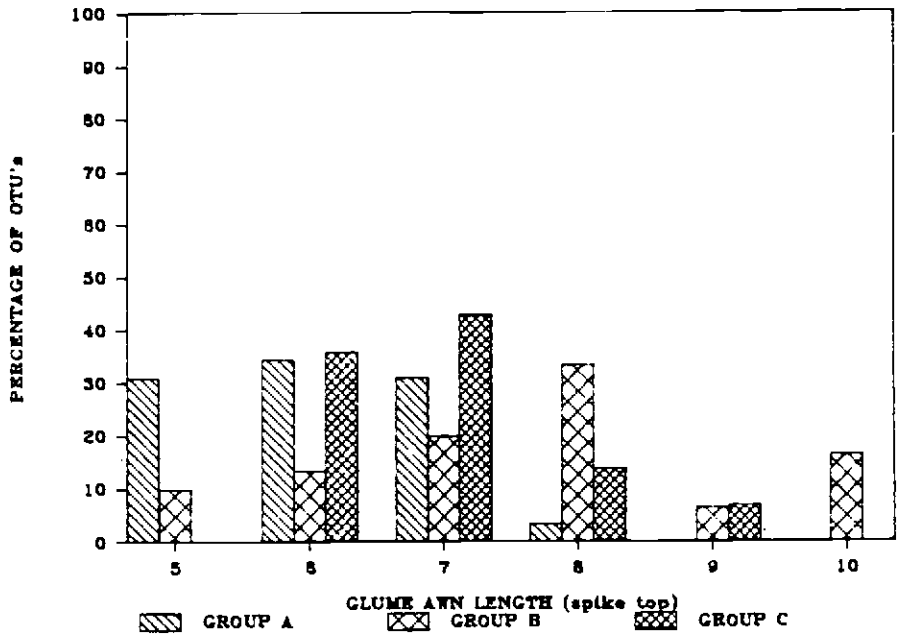
Appendix Table 4. Frequency distribution of hair length (mm) on the abaxial surface in groups A, B and C of T. turgidum ssp. carthlicum.



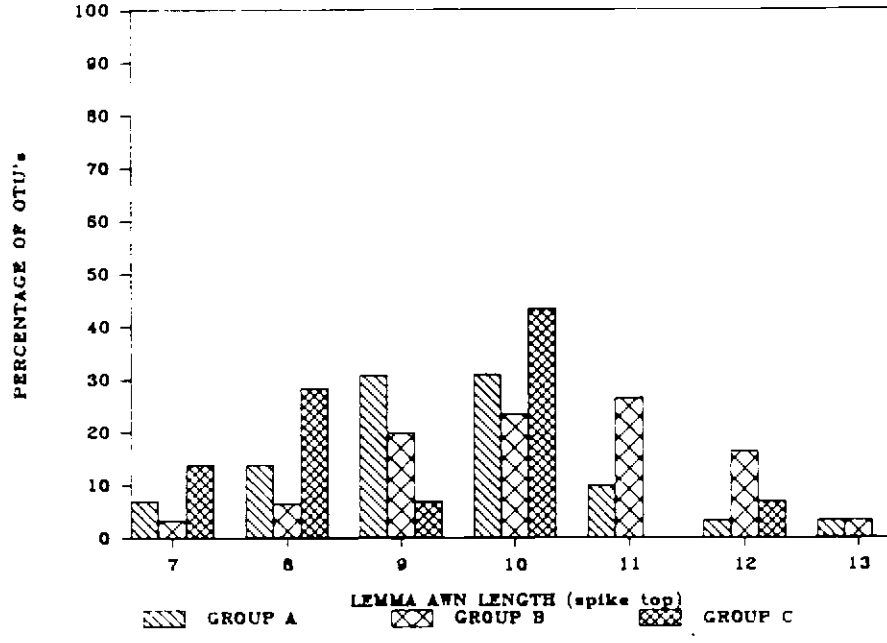
Appendix Figure 5. Histogram of stem solidness in the middle of the second internode (from top to bottom) for groups A, B and C of T. turgidum ssp. carthlicum.



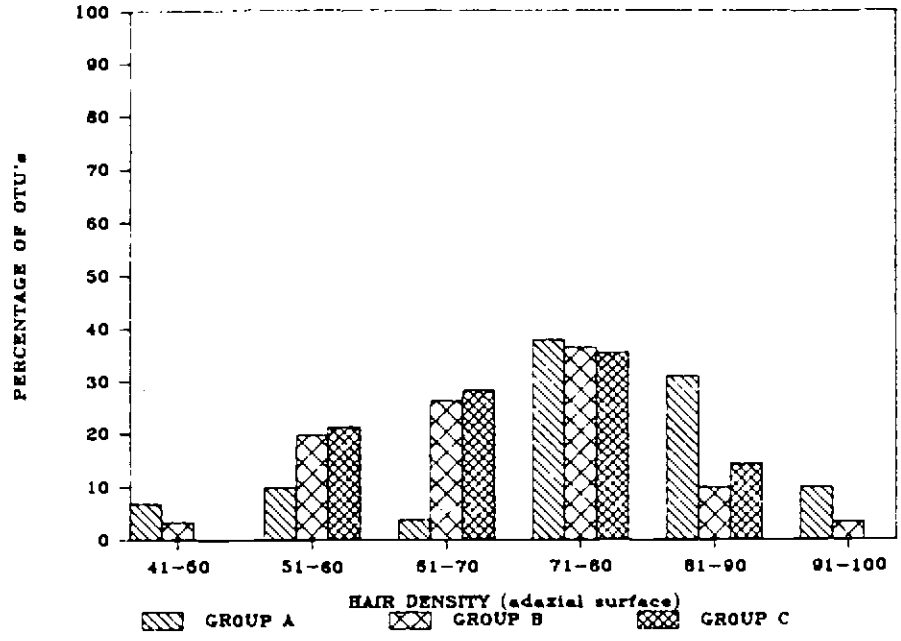
Appendix Figure 6. Histogram of second leaf width (cm) for groups A, B and C. of T. turgidum ssp. carthlicum.



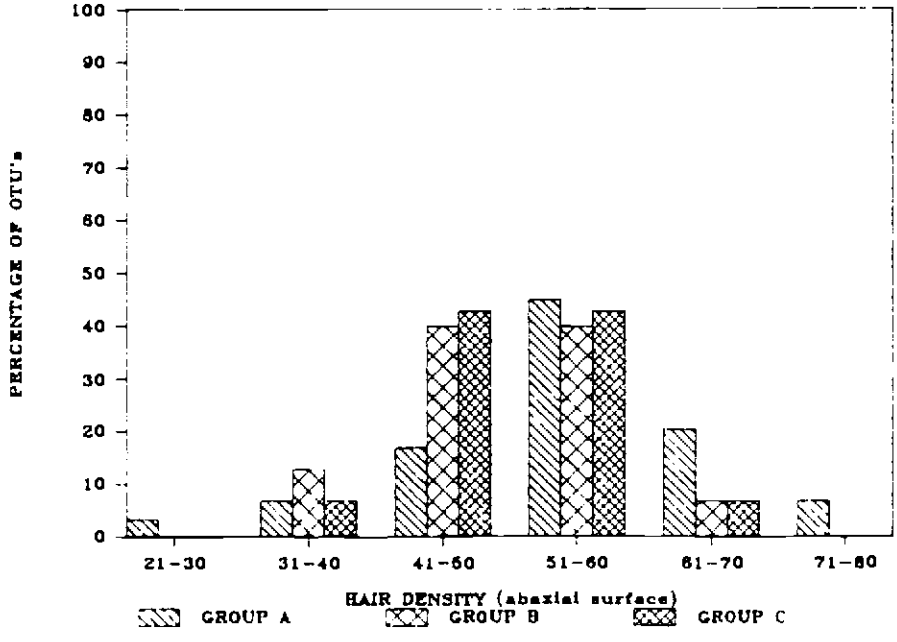
Appendix Figure 7. Histogram of glume awn length (cm) on the spike top for groups A, B and C of T. turgidum ssp. carthlicum.



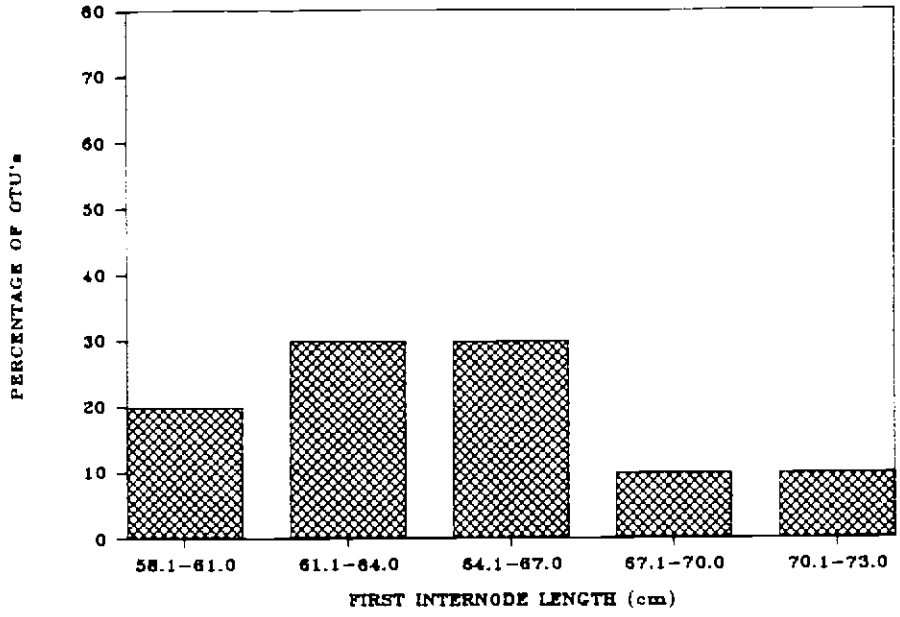
Appendix Figure 8. Histogram of Lemma awn length (cm) on the spike top for groups A, B and C of T. turgidum ssp. carthlicum.



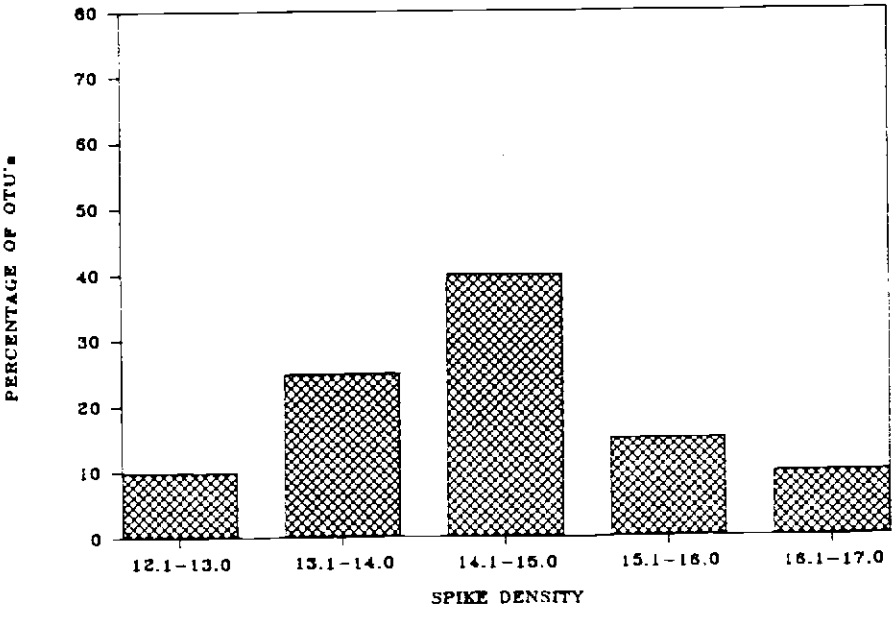
Appendix Figure 9. Histogram of hair density on the adaxial surface for groups A, B and C of T. turgidum ssp. carthlicum.



Appendix Figure 10. Histogram of hair density on the abaxial surface for groups A, B and C of T. turgidum ssp. carthlicum.



Appendix Figure 11. Frequency distribution of first internode length (cm) for group 1 (ssp. carthlicoides).



Appendix Figure 12. Frequency distribution of spike density for group 1 (ssp. carthlicoides).