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Awn anatomy of common wheat (*Triticum aestivum* L.) and its relatives

Xing-feng[1,2] Li, Du Bin[2] and Wang Hong-gang[1, 2*]

[1]State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an, 271018 China
[2]Agronomy College, Shandong Agricultural University, Tai'an, 271018 China

Abstract — Awn of wheat is an important photosynthetic and transpiration organ on spike. In this study, awn transverse sections of *Triticum monococcum*, *Triticum dicoccum*, *Triticum durum*, *Secale cereale*, *Hordeum vulgare* and 3 common wheat cultivars, a synthetic hexaploid wheat were observed. Epidermal, chlorenchyma, sclerenchyma, and conducting tissues were presented in all observed cross-section of awns, difference between wheat and its relatives in awn anatomy were also observed. The cluster result using 16 awn anatomical traits is consistent with the results of traditional morphological traits, which indicated that using awn anatomical characters to distinguish species and to analyze the genetic relationship is feasible.

Key Words: Anatomy; Awn; Genetic relationship; Wheat.

INTRODUCTION

Awn, the long slender extension of lemma in wheat and barley, has long been of interest for centuries (Zoebl and Mikosch 1892). It plays an important role in protection against animals and as a mechanism of seed dispersal. Awns of wild wheat guide a ripe grain to the earth with the pointed end downwards by providing it with the correct balance as it falls, and they are also able to propel the seeds on and into the ground. The arrangement of cellulose fibrils causes bending of the awns with changes in humidity, silicified hairs that cover the awns allow propulsion of the unit only in the direction of the seeds (Elbaum *et al*. 2007).

Awn also plays a dominant role as an important transpiration and photosynthetic organ in ear. It possesses a large surface area, sometimes can equal that of the ground surface, and can exceed that of the flag leaf blade in *Triticum durum* (McDonough and Gauch 1959). It can work well at the time of heading, while some of the leaves are already senescent or heavily shaded. The pathway for assimilation movement from awns to the kernels is minimal (Evans *et al.* 1972), which makes awns an ideally place for light interception and CO_2 uptake. Study of oxygen evolution and PEPCase activity assays of flag leaves and awns revealed that the rate of photosynthesis decrease much earlier in flag leaves than in awns, the activity of PEPCase was much higher in awns than in flag leaves throughout ontogeny; And the value was particularly high at the late stages of grain filling (Li *et al.* 2002; Li *et al*. 2006). These rusults indicated that awn also plays a dominant role in contributing to large grains and a high grain yield in awned wheat cultivars, particularly during the grain-filling stages.

In barley, a recently study showed that the awn preferentially expressed genes for photosynthesis, the biosynthesis of chlorophyll and carotenoids, and reactive oxygen species scavenging, while the lemma and palea overexpressed defense-related genes compared with the awn (Abebe *et al.* 2009). This suggests the lemma and palea are mainly protective organs,

*Corresponding author: phone: 0086-538-8242141; fax: 0086-538-8242226; e-mail: lixf@sdau.edu.cn

whereas the awn is primarily a photosynthetic organ. It provides molecular evidence that awn is the major photosynthetic organ of the spike. The presence of awns can double the rate of net ear photosynthesis (EVANS and RAWSON 1970), it contribute about 40-80% of the total spike carbon exchange rate, depending on the species (GRUNDBACHER 1963; DAS and MUKHERJEE 1991; WEYHRICH 1995; BLUM et al. 1999; KHALIQ *et al*. 2008; MOTZO and GIUNTA 2008; BALKAN and GENÇTAN 2009).

The awn anatomy of wheat and barley has been described in some studies (GRUNDBACHER 1963; WANG *et al*. 1993; PONZI and PIZZOLONGO 2005), however, no studies have focused on difference and diversity of awns among wheat varieties and wheat relatives. In this study, the transverse section of awns in common wheat varieties and wheat relatives were studied, the phylogenetic relationships among different wheat species were assessed based on the awn anatomy traits.

MATERIALS AND METHODS

Plant Materials - The plant materials used in this study included *Triticum monococcum* L. (AA, 2n = 2x = 14), *Triticum dicoccum* Schubl. (AABB, 2n = 4x = 28), *Triticum durum* Desf. (AABB, 2n = 4x = 42), *Hordeum vulgare* L. (HvHv, 2n = 2x = 14), *Secale cereale* L. (RR, 2n = 2x = 14), a synthetic hexaploid wheat Am3 (AABBDD, 2n = 6x = 42), and three common wheat (*Triticum aestivum* L.) varieties Yumai49, Jimai20, and SN051-1 (AABBDD, 2n = 6x = 42). Am3 is synthesized by crossing *Triticum carthlicum* Nevski (AABB, 2n = 4x = 28) with *Aegilops tauschii* Cosson *(DD, 2n = 2x=14)*, which was obtained from Crop Institute, Chinese Academy of Agricultural Sciences. The others materials used in this study were preserved by our laboratory.

Analysis of awn sections - Ten days after the anthesis, awns (Fig. 1a, 1b) from 2 or 3 plants were collected from spike and immediately fixed in 2.5% glutaraldehyde solution in 0.1 M sodium phosphate buffer (pH 7.0) overnight at room temperature, post-fixed with 1% (w/v) osmium tetroxide in phosphate buffer at 4°C, and then embedded in Epon812 (Shell Chemical, Houston, TX, USA) following a standard dehydration procedure. Transverse sections (about 1 cm from the base of the awn, Fig.1b), 2.5 μm thick, were cut with a LKB-V microtome, and then stained in 1% (w/v) toluidine blue in 1% (w/v) disodium tetraborate, and observed under an optical microscope (Olympus BX-51, Japan) with automatic camera.

Data analysis - Sixteen anatomical characters were observed and measured on cross section of awn (some tissues and characters were showed in Fig. 1c): shape of transverse section, angle of abaxial terminal, left angle and right angle of adaxial terminal, edge shape in the adaxial terminal, lengths in the longitudinal axis, lengths in the lateral axis, the ratio between lengths in the longitudinal axis and the lateral axis, row

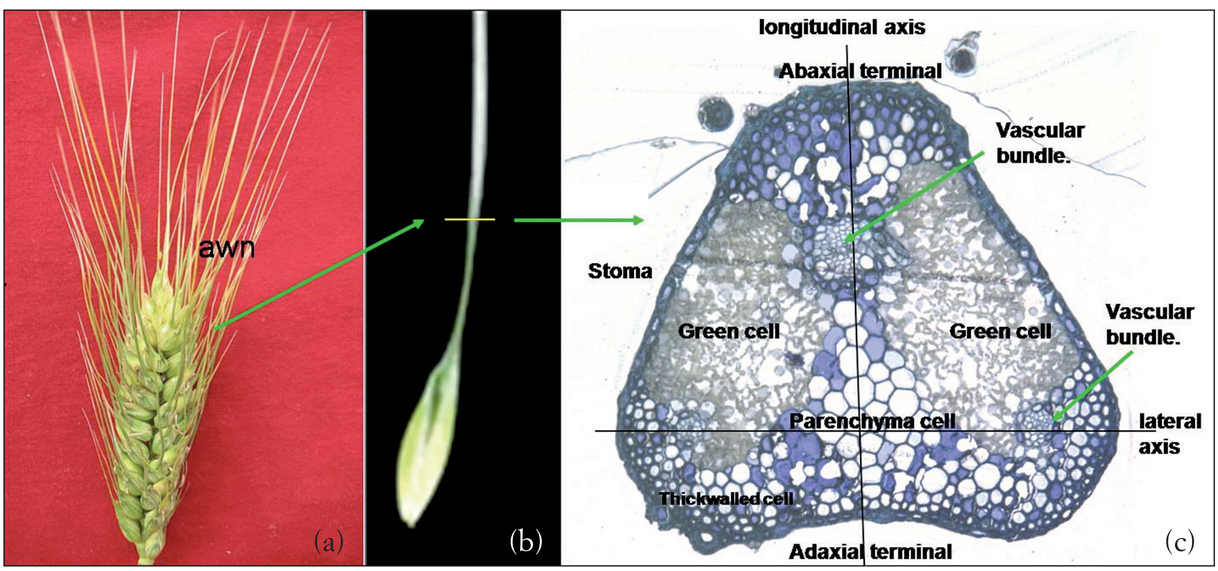

Fig. 1 — Awn and its cross-section of common wheat. (a) Spike and awn of common wheat. (b) Position of the observed cross-section on the awn. (c) Some tissues and measured characters of the awn.

TABLE 1 — Different characteristics of cross-section of awn in wheat and its relatives

Characters	Yumai49	Jimai20	SN051-1	*T. monococcum*	*T. dicoccum*	*T. durum*	Am3	Barley	Rye
Shape of transverse section*	1	1	1	1	1	1	1	2	1
Angle of abaxial terminal	55.0	62.0	56.0	50.0	35.0	40.0	58.0	120.0	50.0
Left angle of adaxial terminal	65.0	66.0	64.0	70.0	80.0	80.0	62.0	30.0	35.0
Right angle of adaxial terminal	60.0	51.0	60.0	60.0	65.0	60.0	60.0	30.0	25.0
Edge shape in the adaxial terminal**	1	2	2	3	3	2	2	3	1
Length in the longitudinal axis (mm)	0.92	1.04	1.02	1.15	0.95	1.32	1.00	0.55	0.75
Length in the lateral axis(mm)	1.04	1.05	1.13	1.12	0.86	1.04	1.05	1.59	0.90
Length in the longitudinal axis / Length in the lateral axis	0.885	0.990	0.903	1.027	1.105	1.269	0.952	0.346	0.833
Row numbers of stomata on either side of epidermis	4	4	4	2	2	5	4	2	2
Vascular numbers	3	3	3	3	3	3	3	3	1
The ratio between the distance of large vascular bundles to the adaxial t erminal and to the abaxial terminal	2.577	2.281	1.684	1.556	2.167	3.125	1.857	1.037	1.500
Layer number of parenchyma cells between two green cell tissues	3	3	3	3	3	3	3	6	0
Layer number of parenchyma cells between green cell tissue and epidermis	1	1	1	1	2	1	2	2	1
Area of green cell tissue(mm^2)	0.25	0.24	0.27	0.23	0.15	0.26	0.25	0.13	0.18
Total area (mm^2)	0.79	1.00	0.75	0.94	0.88	0.99	0.77	0.41	0.57
Green cell area / total area	31.14	24.20	36.39	24.46	17.31	25.71	32.64	32.36	31.75

*: 1, Acute triangle; 2, Obtuse triangle
**: 1, concave; 2,flat; 3,convex

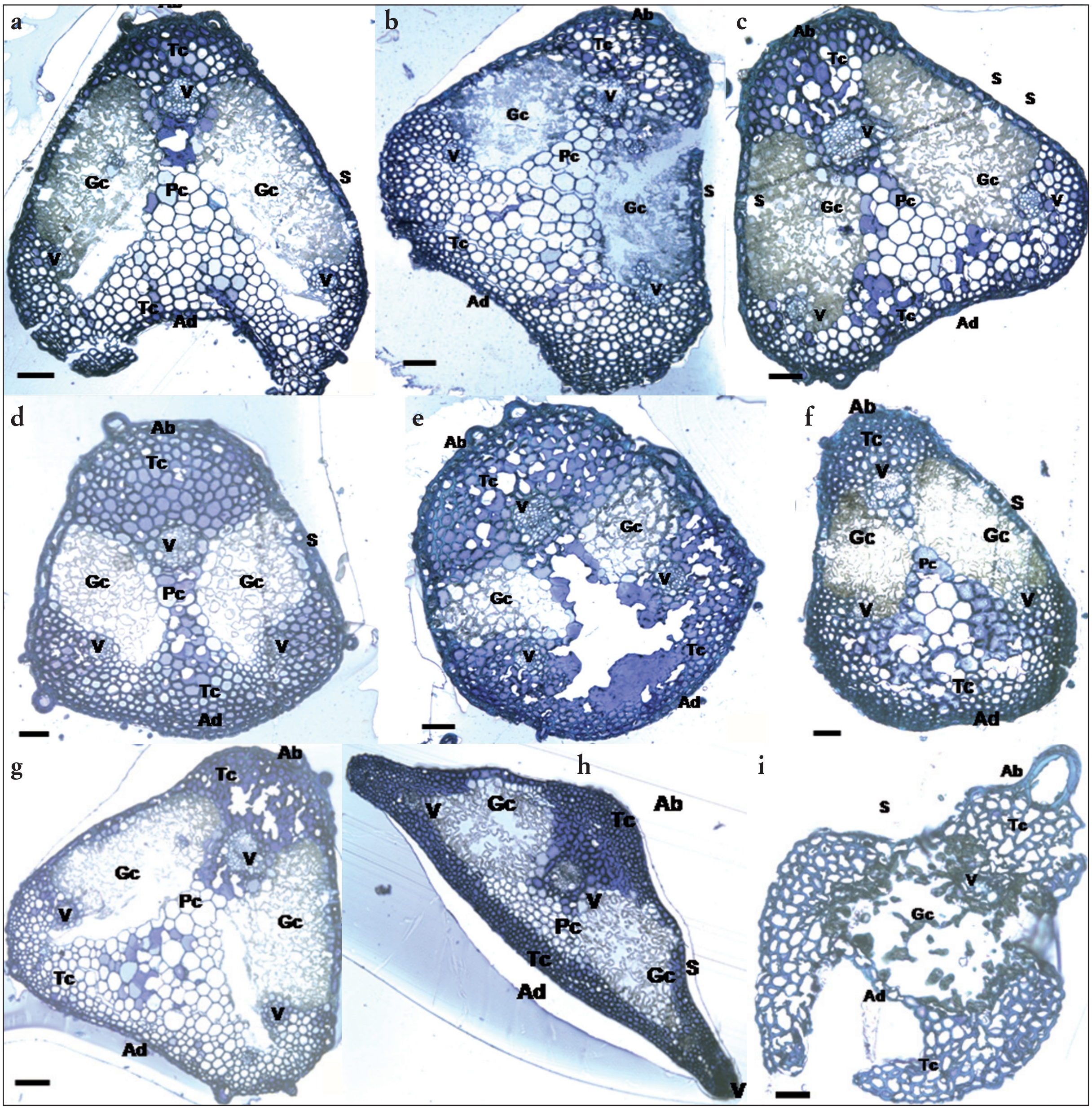

Fig. 2 — Awn cross-section of common wheat and its relatives. (a) Yumai49. (b) Jimai20. (c) SN051-1. (d) *T. monococcum*. (e) *T. dicoccum*. (f) *T. durum*. (g) Synthetic hexaploid wheat. (h) Barley. (i) Rye. **Note:** Ab. Abaxial terminal, Ad. Adaxial terminal, Gc. Green cell, S. Stoma, Pc. Parenchyma cell, Tc. Thickwalled cell, V. Vascular bundle. Scar = 50 μm.

numbers of stomata on either side of epidermis, vascular numbers, the ratio between the distance of large vascular bundles to the adaxial terminal and to the abaxial terminal, layer numbers of parenchyma cells between two green cell tissues, layer numbers of parenchyma cells between green cell tissue and epidermis, area of green cell tissue, area of whole transverse section, and the ratio of area of green cell tissue to total area. Two plates of transvers section from different awn were used for analysis, data showed in table 1 was the average. The lengths, areas and angles were calculated by using ImageJ software (http://rsb.info.nih.gov/ij).

To examine the genetic relationship among wheat and its relatives, Euclid genetic distance was generated by SIMILARITY based on 16 analysis characters and a dendrogram was constructed with the unweighted pair-group method of averages (UPGMA) with 1,000 permutations of bootstrapping NTSYS-pc software version 2.1 (Rohlf 2000).

RESULTS

Basic tissue structure of transverse section of awn - Figures about cross-section of awns were displayed in Fig. 2-a to Fig. 2-i.

From the cross-section observation (Fig. 1c and Fig. 2), four types of tissues are present in cross-section of awn: epidermal, chlorenchyma, sclerenchyma, and conducting tissues. The epidermal tissue consisted of long, narrow thick-walled cells and small oval or square cells as the papillae bump, and also short, thick-walled and fine-tipped single-cell known as hair which oblique forward and make the awn surface rough. Rows of stomata were found along either side of epidermis on abaxial terminal, whereas no stomata presented on adaxial terminal. Under the stomatic band there were two strands of chlorenchyma tissues, or green cell tissue which were differentiated from the parenchyma cells. Mature green cells were irregular, and there were many interstices between them, it would be benefit for the gas exchange of the awn. There were zonal distribution of sclerenchyma tissue under the epidermis, which was made of thick-walled cells and would be important for the awn structure and avoiding water transpiration. Three vascular bundles always presented in each awn, a large central one with complete structure at abaxial terminal and two small ones at adaxial terminal, they are important for the water and assimilation movement.

Difference among wheat and its relatives in awn anatomy - Results of the 16 awn anatomical characters were listed in Table 1.

By comparing the angle of abaxial terminal, left angle and right angle of adaxial terminal, most awn transverse section of wheat and its relatives have acutely triangular form, except that of *Hordeum vulgare* was obtusely triangular (Fig. 2-h).

Transverse section of awn can be divided into three types based on the edge shape in the adaxial terminal: the concave type was presented in Yumai49 and *S. cereale*, the flat type presented in *T. durum*, Jimai20, SN051-1 and Am3, and the convex type presented in *H. vulgare*, *T. monococcum* and *T dicoccum*.

By measuring the lengths in the longitudinal axis, lengths in the lateral axis, and the ratio between them, awn cross-sections of *T. monococcum*, *T. dicoccum* and *T. durum*, Yumai49, Jimai20, SN051-1, Am3 and *S. cereale* look like round because the ratio of them were close to 1.0, while the ratio in *H. vulgare* was only 0.346 which looks very flat.

There are four rows of stomata on either side of epidermis in Yumai49, Jimai20, SN051-1,

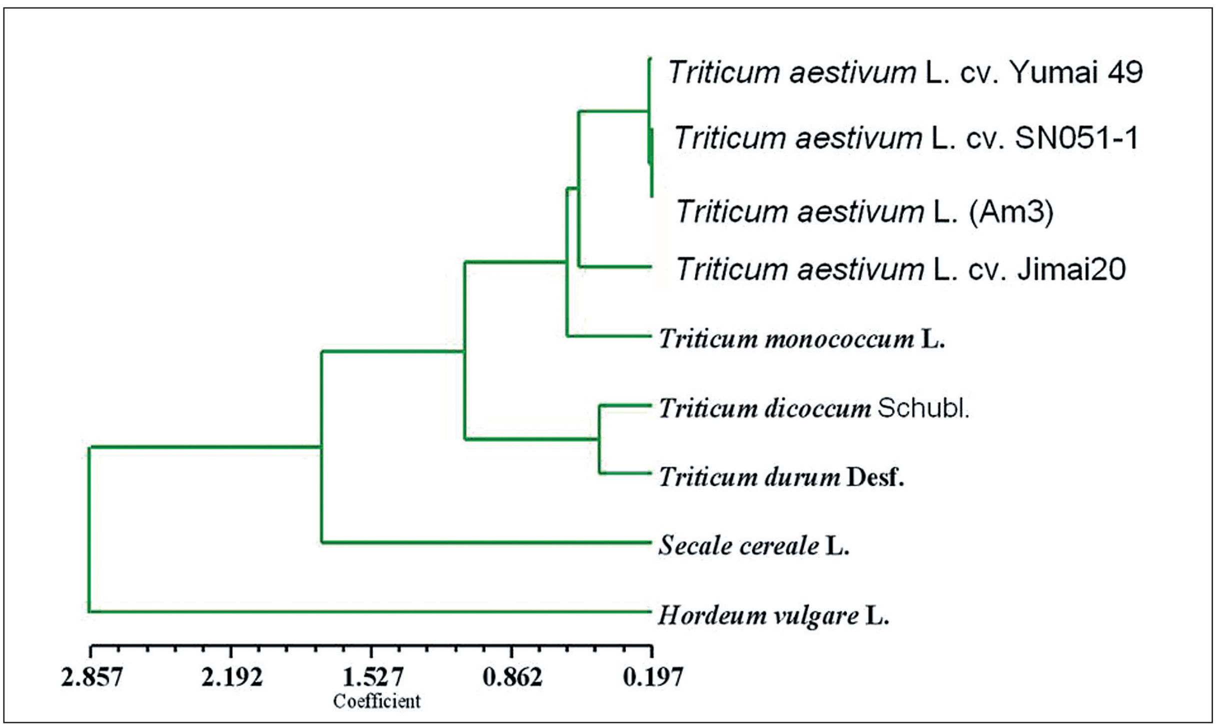

Fig. 3 — Dendrogram obtained by UPGMA cluster based on data from transverse section of awn.

Am3, and two rows in *S. cereale*, *T. monococcum*, *T. dicoccum* and *H. vulgare*, and five rows in *T. durum*.

Most observed materials in this study had three vascular bundles except for *S. cereale* only had one vascular bundle. The ratio between the distance of large vascular bundles to the adaxial terminal and to the abaxial terminal of *H. vulgare* was 1.037, while the ratio of other materials were larger than 1.500, which means that the large vascular bundles located in the central of the awn while it located near the abaxial terminals in other materials.

Most materials had three layers of parenchyma cells between two green cell tissues, except six layers in *H. vulgare* and no parenchyma cells in *S. cereale*. Only one layer of parenchyma cell between green cell tissue and epidermis presented in Yumai49, Jimai20, SN051-1, *T. monococcum*, *T. durum* and *S. cereal,* while two layer of parenchyma cells presented in Am3, *T. dicoccum* and *H. vulgar*.

Total surface area of awn cross-section of *H. vulgare* and *S. cereale* were obviously lower than those of the other materials. While compared the ratio of green cell tissue area to total surface area, the ratio of Jimai20, *T. monococcum*. and *T. durum* were between 25% to 30% which were higher than *T. dicoccum* (17.31%), and the other materials was higher than 30%.

Genetic relationship among wheat and relatives - An attempt to study the genetic relationship among the species was conducted based on the awn anatomy traits. Phylogenetic dendrogram showing relationships among wheat varieties and its relatives was obtained by UPGMA cluster (Fig. 3).

H. vulgare was firstly in a separate cluster from other materials, secondly *S. cereale* also formed a subcluster showing less similarity with other materials studied, and next *T. dicoccum* and *T. durum* formed a subcluster, *T. monococcum* showed closer genetic relationship with Am3 and three wheat varieties. These hexaploid wheats were separated into two sub-groups, Yumai49, SN051-1 and Am3 grouped in one subgroup, and Jimai20 formed another one.

DISCUSSION

In this study, awn transverse section of 3 common wheat cultivars and a synthetic hexaploid wheat, *Triticum monococcum*, *Triticum dicoccum*, *Triticum durum*, *Secale cereale*, *Hordeum vulgare* were studied, the possibility of using the awn anatomy traits to assess phylogenetic relationships were also discussed.

Wheat awn had the same tissues and structure with its relatives, however, there have some difference which could be used to identify species. As shown in this study, *H. vulgare* awn was obtusely triangular compared with the other materials with acutely triangular. Difference on the edge shape in the adaxial terminal could also be used to distinguish species, concave type was found in *S. cereale*, flat type was found in *T. durum* and most wheat varieties, convex was found in *H.vulgare*, *T. monococcum* and *T. dicoccum*. And there was only one vascular bundle presented in the awn of *S. cereal*. Even in the wheat varieties there are also differences of internal structure and layout, the ratio of green cell tissue area to total area in Yumai49 and Jimai20 were 31.14% and 24.2%, respectively.

Morphology characters of stem, leaf and spikelet were usually used to to investigate genetic relationship and identify species (GUO *et al.* 1985). Leaf antomy has proved to be a good phylogenetic tool for grass systematics. Many researchers have succeeded in using leaf antomy to circumscribe species and infer phylogenies (BROWN 1958; COLUMBUS 1999). Culm anatomy in grasses also has been regarding to the usefulness of its characters in phylogenetics (CENCI *et al*. 1984; RAMOS *et al*. 2002).

We attempt to apply these different data in transverse section of awn to analysis genetic relationship of wheat and its relatives. The cluster result indicated that *T. aestivum* with same chromosome constitution AABBDD basically were classified into one group, other species having the close to remote genetic relationship with wheat were *T. monococcum*, *T. dicoccum* and *T. durum*, *S. cereale*, last *H. vulgare*. which is basically consistent with the results of traditional morphological taxonomy (GUO *et al.* 1985) and taxonomy based on the molecular markers (YU *et al*. 2001). Which proved that using awn anatomical characters to distinguish species and to analyze the genetic relationship between wheat and its relatives is feasible.

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REFERENCES

ABEBE T., WISE R.P. and SKADSEN R.W., 2009 — *Comparative transcriptional profiling established the awn as the major photosynthetic organ of the barley spike while the lemma and the palea primarily protect the seed*. The Plant Genome, 2(3): 247-259.

BALKAN A. and GENÇTAN T., 2009 — *The effects of some shotosyntehsis organs on yield components in bread wheat*. Journal of Tekirdag Agricultural Faculty, 6(2): 137-148.

BLUM A., ZHANG J. and NGUYEN H.T., 1999 — *Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production*. Field Crop Research, 64: 287-291.

BROWN W.V., 1958 — *Leaf anatomy in grass systematics*. Botanical Gazette, 119: 170-178.

CENCI C.A., GRANDO S. and CECCARELLI S., 1984 — *Culm anatomy in barley (Hordeum vulgare)*. Canadian Journal of Botany, 62(10): 2023-2027.

COLUMBUS J.T., 1999 — *Morphology and leaf blade anatomy suggest a close relationship between Bouteloua aristidoides and B. (Chondrosium) eriopoda (Gramineae: Chloridoideae)*. Systematic Botany, 23: 467-478.

DAS N.R. and MUKHERJEE N.N., 1991 — *Grain yield contribution by leaf and awn in dwarf wheat (Triticum aestivum L.) after rice (Oryza sativa L.)*. Environment and Ecology, 9: 33-36.

ELBAUM R., ZALTZMAN L., BURGERT I. and FRATZL P., 2007 — *The role of wheat awns in the seed dispersal unit*. Science, 316: 884-886.

EVANS L.T., BINGHAM J., JACKSON P. and SUTHERLAND J., 1972 — *Effect of awns and drought on the supply of photosynthate and its distribution within wheat ears*. Annals of Applied Biology, 70: 67-76.

EVANS L.T. and RAWSON H.M., 1970 — *Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat*. Australian Journal of Agricultural Research, 23: 245-254.

GRUNDBACHER F.J., 1963 — *The physiological function of the cereal awn.* Botanical Review, 29: 366-381.

GUO P.C., WANG S.J. and LI J.H., 1985 — *Studies on morphological evolution, classification and distribution of the Triticeae in China*. Acta Phytotaxonomica Sinica, 23(3):161-169.

KHALIQ I., IRSHAD A. and AHSAN M., 2008 — *Awns and flag leaf contribution towards grain yield in spring wheat (Triticum aestivum L.).* Cereal Research Communications, 36(1): 65-76.

LI H.B., HU Y.X., BAI D.Z., KUANG T.Y., ZHOU F. and LIN J.X., 2002 — *Comparison of chloroplast ultrastructure and 77 Kfluorescence emission spectra between awns and flag leaves in wheat.* Journal of Chinese Electron Microscopy Society, 21: 97-101.

LI X.J., WANG H.G., LI H.B., ZHANG L.Y., TENG N.J., LIN Q.Q., WANG J., KUANG T.Y., LI Z.S., Li B., Zhang A.M. and Lin J.X., 2006 — *Awns play a dominant role in carbohydrate production during the grain-filling stages in wheat (Triticum aestivum).* Physiologia Plantarum, 127: 701-709.

MCDONOUGH W.T. and GAUCH H.G. 1959 — *The contribution of the awns to the development of the kernels of bearded wheat*. Maryland Agricultural Experiment Station Bulletin, 103: 1-16.

MOTZO R. and GIUNTA F., 2008 — *Awnedness affects grain yield and kernel weight in near-isogenic lines of durum wheat.* Australian Journal of Agricultural Research, 53(12): 1285-1293.

PONZI R. and PIZZOLONGO G., 2005 — *Cytological and anatomical observations on the awn and lemma of wheat (Triticum aestivum L. cv. Ofanto)*. Plant Biosystems, 139(3): 345-348.

RAMOS J.C.; TIVANO J.C. and VEGETTI A.C., 2002 — *Anatomical studies of reproductive stems of Bromus auleticus Trin. ex Ness (POACEAE)*. Gayana Botany, 59(2): 51-60.

ROHLF F.J., 2000 — *NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System*, Version 2.1., New York, USA.

VERVELDE G.J., 1953 — *The agricultural value of awns in cereals*. Netherlands Journal of Agricultural Science, 1: 2.

WANG Z., GU Y.J. and GAO Y.Z., 1993 — *Structure and photosynthetic characteristics of awns of wheat and barley.* J. Integr. Plant Biol., 35(12): 921-928.

WEYHRICH R.A., CARVER B.F. and SMITH E.L., 1995 — *Effect of awn suppression on grain yield and agronomic traits in hard red winter wheat.* Crop Science, 34: 965-969.

YU B.L., HUANG Z.F., ZHOU W.J. and ZHANG W.J., 2001 — *Evolution study of wheat (Tritium aestivum L.) A, B and D genome based on DNA sequence similarity.* Acta Genetica Sinica, 28(7): 635-639.

ZOEBL A. and MIKOSCH C., 1892 — *Die Function der Grannen der Gerstenähren*. Bot. Centralbl., 54: 240.

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