Brief-Report

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Involucral Bracts Anatomy in the Capitulum of Primitive Strains and Modern Genotypes of Sunflower (*Helianthus annuus* L.)

Abstract: The anatomy of involucral bracts (IB) of the capitulum was studied in two sunflower domesticated primitive genotypes (*Helianthus annuus* L.), Havasupai and Hopi, HA89B line and DKOP3845 hybrid. Stomata and trichomes were counted on the adaxial and abaxial epidermis. In all cases the IB showed an one-layered adaxial and abaxial epidermis, secretory ducts and parenchymatic cells with abundant chloroplast. The vascular system was similar to that of the foliage leaves; however, their bundles were smaller, with an abaxial surface with higher abundance of glandular and non-glandular trichomes and with the presence of stomatas. IB of Havasupai and Hopi showed higher number of adaxial hypodermic strata than those of HA89B and DKOP3845 (4 vs. 1) and one mesophyllum with inverted polarity with respect to a foliage leaf: the presence of a spongy parenchyma on the adaxial side was observed with a rudimentary palisade parenchyma on the abaxial side. Stomatal density of the IB was significantly higher in Hopi and Havasupai than in HA89B and DKOP3845, with values ranging from 132 to 156 vs. 73 to 110 stomata/mm^2 respectively. It is concluded that from the functional point of view, sunflower breeding produced undesired changes in the IB anatomy.

Keywords: sunflower, involucral bracts, primitive strains genotypes, modern genotypes, reverse polarity

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Introduction

Contribution to CO_2 fixation by non-foliar organs, such as reproductive structures (Weiss et al. 1988; Blanke and Lenz 1989), stem tissues (Nilsen 1995; Pfanz and Aschan 2001) and even roots (Kitaya et al. 2002), has been demonstrated by several authors. The involucral bracts (IB) of the sunflower (*Helianthus annuus* L.) capitulum constitute an example of highly modified photosynthetizing organs that contribute to the photosynthesis budget during the generation of yield. Laxman and Srivastava (2000b) compared the IB photosynthetic and respiratory activity with that of foliage leaves in two genotypes of sunflower at different growth stages. During ontogeny, the bract net Ribulose bisphosphate carboxylase (RuBP Case) activity increased gradually until seed filling. But the RuBP Case activity of IB was 10 to 14 times lower than in the foliage leaves. The lower photosynthetic rate of bracts relative to foliage leaves was due to lower stomatal conductance (Laxman and Srivastava 2000a) and low chlorophyll concentration due to the absence of palisade cells (Laxman and Srivastava 2000b). They also found that PEP carboxylase and NADP-malate dehydrogenase activity in bracts was higher than in foliage leaves, which indicated the possibility of involvement in refixation of respiratory released carbon (Laxman and Srivastava 2000b).

The aim of the present work was to identify the morphological changes occurring in a highly modified photosynthetizing organs in sunflower such as the IB as a result of its genetic improvement, in order to consider or discard their incorporation as a morphological feature that may be improved in future breeding programs.

Materials and methods

Two domesticated primitive sunflower genotypes Havasupai and Hopi, the line HA89B and the commercial hybrid Dekasol OilPlus (DKOP) 3845, were sown on October 2013 at the experimental field of the Agronomy Department UNSur, Bahía Blanca, Argentina (38° 45′ Lat. S; 62° 11′ Long. O). The soil was a Typic *Ustipsamment* (Soil Survey Staff 1999). At growth stage V4 (plant with 4 foliage leaves; Schneiter and Miller 1981) plant density was adjusted to 5.6 plants/m^2. Fertilization with potassium nitrate (NO_3K, 15%N), at a rate of 60 Kg N/ha was conducted at sowing time and at anthesis (stage R5). Soil water content was maintained at optimum levels by drip irrigation during all the crop cycle. Weeds were adequately controlled manually. Plague or disease control was not necessary.

For histological analysis and quantification of stomatas and trichomes in the abaxial and adaxial epidermis, six IB from the external whorl of the capitulum of three plants per genotype were collected at stage R8 (Schneiter and Miller 1981).

For histological study, segments of the medium portion of the three IB per plant were placed in recipients with FAA fixative (formalin–acetic acid–ethanol–water, 10:5:50:35). The tissue segments were processed following conventional histological techniques (Ruzin 1999), embedded in Paraplast (Leica) and cut at 8–10 μm with rotary microtome. They were then stained in safranin–fast green (Ruzin 1999), observed and photographed under a Nikon Labophot-2 microscope equipped with a Nikon Coolpix 4500 digital camera.

To observe and quantify stomatas and trichomes, molds of the abaxial and adaxial epidermis of three IB per plant were taken using the technique described by Hernández and Green (1993). A selected area on each side of the bract was covered with an hydrophilic polyvinyl siloxane impression material (Extrude Wash; Kerr Corporation, USA). Once polymerized, the material was removed with tweezers and painted with a layer of colorless synthetic enamel paint. The enamel copy was dry mounted in a microscope slide and observed and photographed as mentioned above. With the digital images and the ImageJ software (Rasband 2011), three areas of 0.1 mm^2 per image were selected and the stomatas counted, obtaining an estimation of the stomatal density (number of stomatas/mm^2 IB). Data obtained were subjected to analysis of variance and the difference among measurements was compared using the Tukey test with $p \leq 0.05$ (Di Rienzo et al. 2010).

Results

The IB of the domesticated primitive genotypes, Havasupai and Hopi, the HA89B line and the DKOP3845 hybrid showed, the same as foliage leaves (nomophylls), an adaxial and abaxial unstratified epidermis, secretory ducts, parenchymatic cells with abundant chloroplasts, a vascular system with collateral bundle, which were smaller than the foliar ones and an abaxial area with higher abundance of glandular and non-glandular trichomes and stomatas (Figure 1).

The primitive genotypes Havasupai and Hopi showed IB with multistratified adaxial hypodermis (Table 1) and dorsiventral heterogeneous mesophyllum with inverted polarity with respect to foliage leaves: the spongy parenchyma was on the adaxial surface and a rudimentary palisade parenchyma was on the abaxial side (Figure 1(A) and (B)). The phenomenon of reversed polarity has been

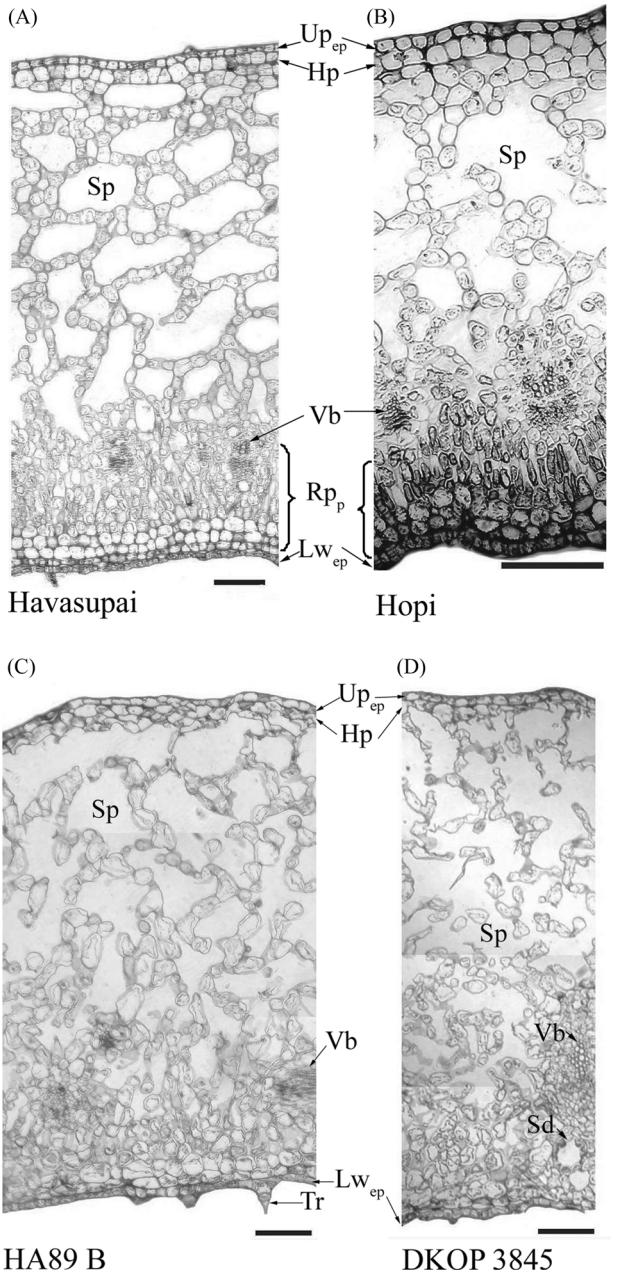

Figure 1: Involucral bracts cross sections of Havasupai (A), Hopi (B), HA89B (C) and DKOP3845 (D). Hp: hypodermis, Lw_{ep}: lower epidermis, RPp: rudimentary palisade parenchyma, Sd: secretory duct, Sp: spongy parenchyma, Tr: trichome, Up_{ep}: upper epidermis, Vb: vascular bundle. Scale: 200 µm.

Table 1: Morphological characters of involucral bracts of the primitive strains and modern genotypes of sunflower studied in this work.

					Morphological characters
Sunflower genotype	**Adaxial hypodermal layers (Figure 1)**	**Adaxial spongy parenchyma**	**Abaxial palisade parenchyma**	**Abaxial stomata* (N^o mm^{-2}) (Figure 2)**	**Abaxial trichomes density**
Havasupai	3/4	YES	YES (Rudiment)	156 a	Ample
Hopi	3/4	YES	YES (Rudiment)	132 ab	Ample
DKOP 3845	1	YES	NO	110 b	Scarce
HA89B	1	YES	NO	73 c	Scarce

Note: *Means followed by a different letter for each row indicates a significant difference (LSD test, $P \leq 0.05$).

reported for vegetative organs in other species such as foliage leaves of mutant *Arabidopsis thaliana* (McConnell and Barton 1998), *Zea mays* Rolled leaf1 (Rld1, Nelson et al. 2002) or *Fabiana imbricata* exposed to direct sunlight or shade (Cosa et al. 2012). IB of HA89B and DKOP3845 showed an one-layered adaxial hypodermis (Table 1) and an homogeneous mesophyllum composed of an spongy parenchyma with no presence of palisade parenchyma (Figure 1(C)–(D)).

Other differences observed between modern and primitive genotypes were due to higher stomatal and trichomes density in Hopi and Havasupai (Figure 2(A))

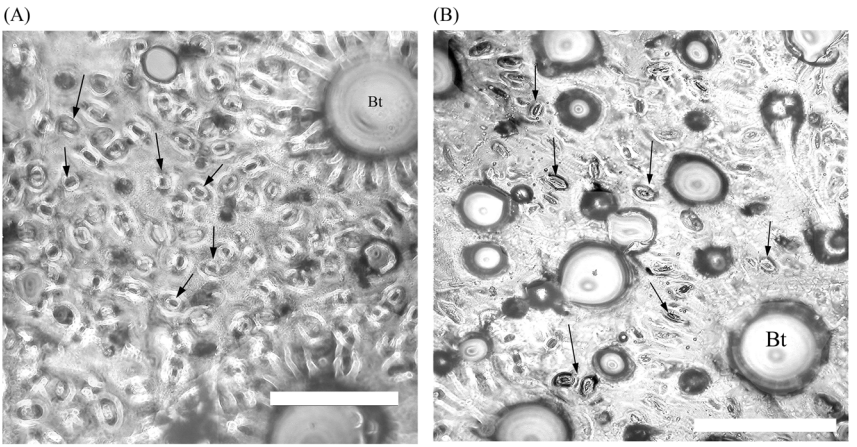

Figure 2: Detail of the abaxial epidermis of the involucral bracts in Havasupai (A) and HA89B (B). Bt: base of a trichome. Arrows indicate stomata. Scale: 200 µm.

than in HA89B (Figure 2(B)) and DKOP3845, with values ranging from 132 to 156 vs. 73 to 110 stomata/mm^2 respectively (Table 1).

Conclusions

The presence of palisade parenchyma and abundant stomata on the side of the IB of primitive genotypes exposed to light (abaxial side) shows an important physiological advantage in this photosynthetically active organ, with respect to the homogeneous mesophyllum present in the IB of modern hybrids. Also the higher density of the stomata in the IB of primitive genotypes could generate increased stomatal conductance, as compared to modern genotypes that have lower stomatal density (Laxman and Srivastava 2000a).

The IB of the domesticated primitive genotypes show a mesophyllum with reverse polarity, possibly due to there response to direct exposure to solar radiation received by the abaxial surface. The devoid of palisade parenchyma during the anatomical development of the IB of modern sunflower may reflect loss of that trait during human selection or may be due to a different cause or set of causes, not studied in this work.

Future studies to compare the physiological (photosynthesis and respiration) and biochemical activity of the RuBP carboxylase among the IB of the primitive, Havasupai and Hopi and, and the modern genotypes will help to define the magnitude of their importance as a morphological trait to be considered in future crop breeding plans.

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