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Full Length Research Paper

Microstructure of hygroscopic awns in three poaceae species

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ABSTRACT

Microstructure of hygroscopic awns in diaspores of *Aristida funiculata*, *Danthoniopsis barbata*, and *Dichanthium foveolatum* (Poaceae) was examined. Hygroscopically active parts of the awns are twisted and sensitive to ambient humidity. These features reflect the fine structure of the cell wall in two distinctive layers of fiber cells, which lead to twisting and untwisting of hygroscopically active parts when they absorb or lose water. Periodic changes in ambient humidity initiate hygroscopic action that causes the diaspore to move across soil surface and drill into soil. The fine barbs on the surface of twisted part prevent awns from pushing themselves out of soil. The geometrically ideal conical shape of the barbs and different wettability between barbs and hygroscopic surface of twisted fibers generate gradients in Laplace pressure and surface energy, respectively, that derive water droplets to the hygroscopic part, then enhance "moisture collection" in conditions of dew formation. These characteristics enhance establishment of the diaspores in suitable soil microsite for germination.

Key words: *Aristida funiculata*, *Danthoniopsis barbata*, *Dichanthium foveolatum*. hygroscopic awns, microfibrils, microstructure.

INTRODUCTION

Hygroscopic movement is a physical behavior occurring in dead tissues of the plant as a result of changes in water content. This movement is created when stress develops in drying tissues in which one part shrinks more than the neighboring part (Elbaum et al. 2008). Hygroscopic movement is common in dispersal of spores in ferns, opening of pine cones, opening of pollen sacs in flowering plants, opening of pods in Fabaceae, opening of seed capsule in ice plant and sesame, dispersal of diaspores (seeds or fruits) in Geraniaceae and Poaceae (Fahn & Werker ,1972; Peart ,1979; van der Pijl ,1982; Gutterman ,1993; Dawson et al., 1997; van Rheede van Oudtshoorn & van Rooyen, 1999; Gutterman, 2002; Elbaum et al., 2008; Abraham & Elbaum, 2013; Elbaum & Abraham, 2014).

A special mechanism of hygroscopic movement known as trypanospermy, which means boring dispersal unit, exists in diasporas of Geraniaceae and Poaceae (van Rheede van Oudtshoorn & van Rooyen, 1999;Gutterman, 1993). Trypanospermic or boring diaspores are equipped with a hygroscopic drilling apparatus consisting of two parts. The lower part is capable of spiral twisting by hygroscopic movement whereas the upper part is usually straight and at right angle to the lower one (van Rheede van Oudtshoorn & van Rooyen, 1999). Changes in moisture causes the spiral portion to twist and untwist, acting like a borer pushing the seed (in the base of the lower part) below soil surface.

Although the general mechanism is the same in diaspores of many species of Geraniaceae and Poaceae (van Rheede van Oudtshoorn & van Rooyen, 1999), the microstructure of hygroscopic part of the diaspore is different among species, since there are differences in habitats and nature of soil (Elbaum et al., 2007). In this context, although species such as *Erodium*, *Geranium*, and *Pelargonium* (Geraniaceae) are well described (Abraham et al., 2012; Abraham & Elbaum, 2013; Jung et al., 2014), species of the Poaceae in general still need to be described. Many genera of Poaceae have trypanospermic behavior with active hygroscopic parts,

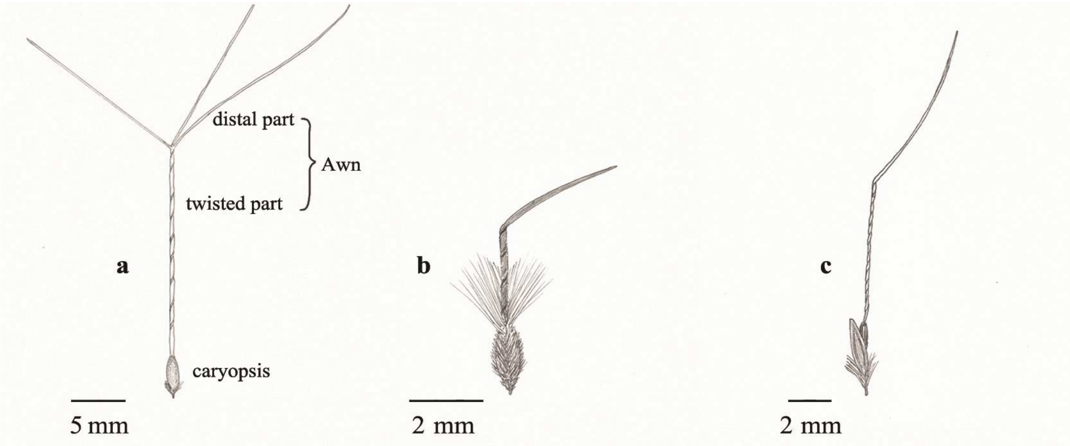

Figure 1. Diaspores of the three studied species. (**a**: *Aristida funiculata*, **b**: *Danthoniopsis barbata*, **c**: *Dichanthium foveolatum*).

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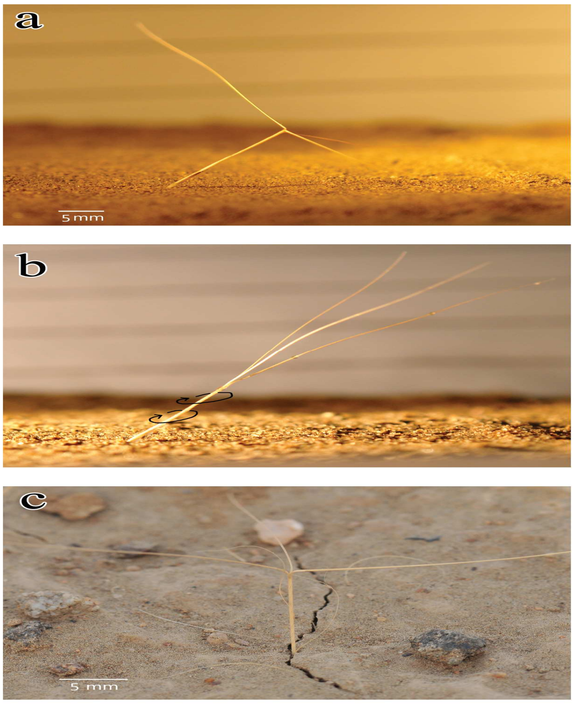

Figure 2.Trypanospermic behavior in diaspore of *Aristida funiculata*. **a**: diaspore on soil in dry state, **b**: after wetting with water spray, **c**: diaspore after repetition of wetting/drying cycles, boring, and establishment of caryopsis in soil crevice.

including *Aristida*, *Stipagrostis*, *Stipa*, *Dichanthium* and *Danthoniopsis*. Most species of these genera inhabit arid and semi-arid regions (Kellogg, 2015).

Diaspores of species in these Poaceae genera consist of two main parts, the caryopsis (with sharply pointed tip) at the base attached to awn (Figure. 1). The awn is divided into two parts, twisted part and distal part. The twisted part is the hygroscopically active part. Tissues of this part entwine anticlockwise when dry. In a damp environment, the hygroscopic tissue strongly imbibes water and the twisted parts expand, rotate the diaspore in clockwise direction, leading to movement that pushes the caryopsis forward crawling through soil surface to a suitable position such as a micro-depressions, a small crevice, or loose sand. Repetition of this movement on a rhythm of ambient humidity or wetting enhances drilling effect by the rotating force produced in the twisted part leading to establishment of the caryopsis in soil at a suitable depth (Figure. 2). This microsite enhances successful germination and seedling establishment.

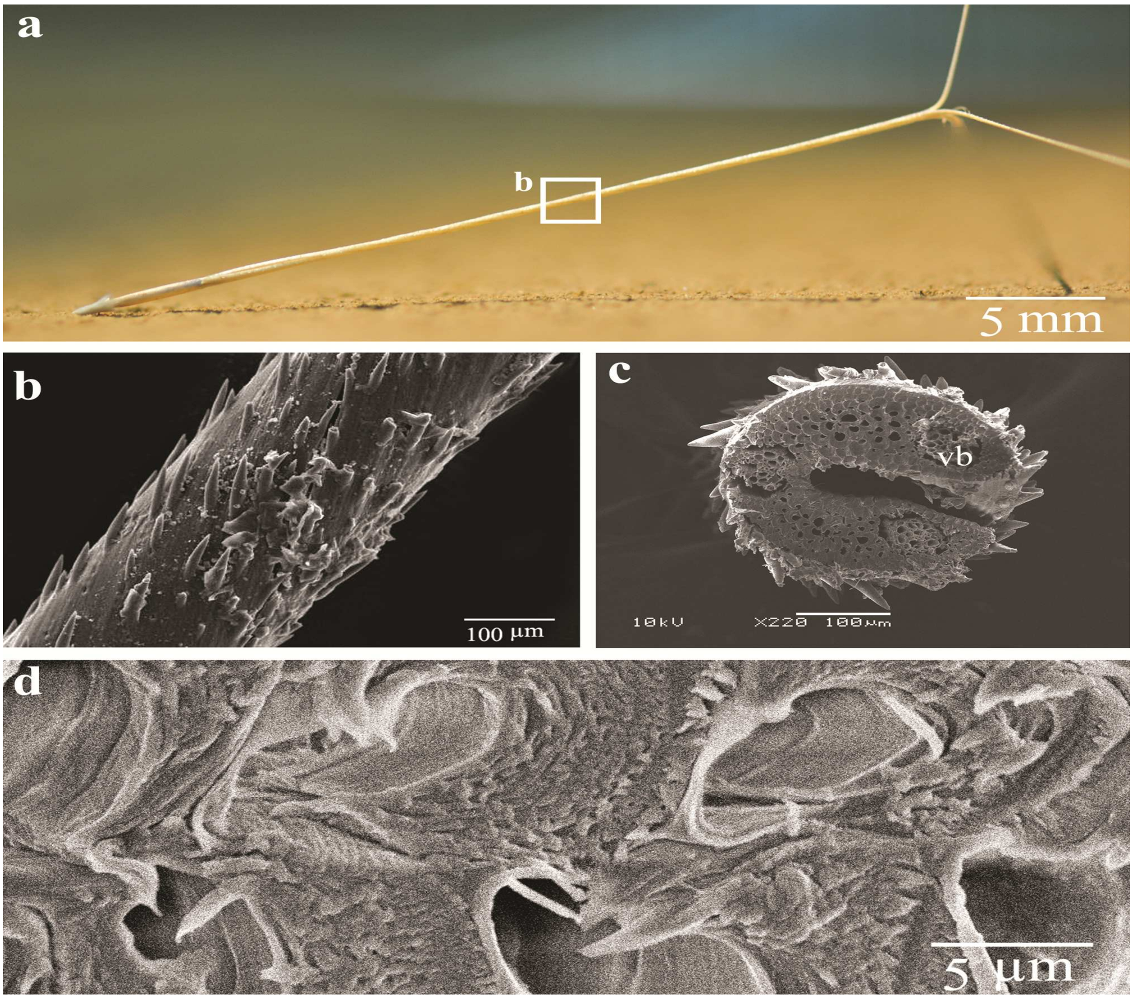

Figure 3. Diaspore of *Aristida funiculata*. **a**: The diaspore, **b**: SEM photo micrograph of twisted part, **c**: SEM photo micrograph of cross section, **d**: SEM photo micrograph of inner layer revealing plywood or spool-like packing of cellulose fibrils in cell walls, **vb:** vascular bundle.

This work aims studying microstructure of the awn hygroscopically active part in *Aristida funiculata* Trin. & Rupr. , *Danthoniopsis barbata* (Nees) C.E. Hubb. and *Dichanthium foveolatum* (Del.) Rob. (Poaceae).

MATERIALS AND METHODS

Diaspores of *Aristida funiculata* and *Dichanthium foveolatum* were collected from their natural habitats in Tihama Hill slopes and coastal area, respectively, in Jazan region (Saudi Arabia). Diaspores of *Danthoniopsis barbata* were obtained from National herbarium, Riyadh, Saudi Arabia. This species inhabits mountainous areas in Southwestern Saudi Arabia (Chaudhary, 1989; Masrahi, 2012).

Cross sections in twisted parts were cut by razor blade after wetting. Dried twisted parts of diaspores were mounted on aluminium stubs and sputter-coated with gold. Samples were examined by SEM (JEOL-JSM 6380 LA, Japan) at 10, 15 and 20 kV.

Characteristics in twisted parts were made on SEM micrographs. Measurements were routinely repeated and standard error was calculated.

RESULTS

Twisted part of the three species revealed the same general structures, including bundle of long fiber cells (in ribbon-like structure) entwined in anti-clockwise direction (dry state) with many silicified antrorse barbs on the surface (Figures. 3-5). Ribbon-like bundles were twisted almost tightly, except in *Danthoniopsis barbata* where the ribbon-like bundles were twisted with large cleavages between the coils (Figures. 4a,b). Barbs were also found on both inner and outer surfaces in this species (Figureures 4b,c). Cross sections showed two distinctive

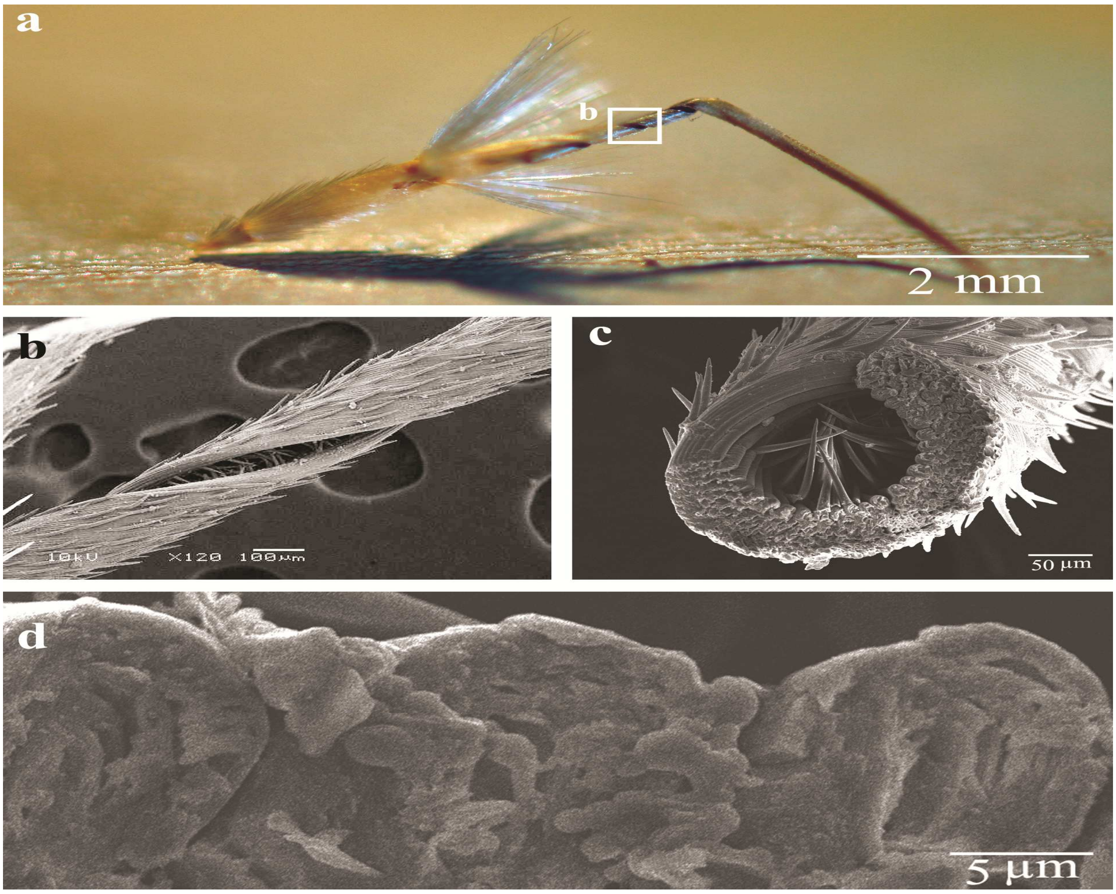

Figure 4. Diaspore of *Danthoniopsis barbata*. **a**.The whole diaspore. **b**. SEM photomicrograph of twisted part . **c**. SEM photomicrograph of cross section. **d**. SEM photomicrograph of inner layer revealed plywood or spool-like packing of cellulose fibrils in cell walls.

layers of fiber cells (Figures. 3-5c), an outer layer of small, thick walled with narrow lumen or completely filled lumen cells, and an inner layer with large, empty lumen cells. Cross sections in twisted part of *Aristida funiculata* revealed big fiber cells between two layers of small, filled lumen fibers. Three vascular bundles were also distributed within the layers (Figure. 3c). Sections in twisted part of *Dichanthium foveolatum* showed two distinctive layers of fiber cells, an outer with small-thick cells and an inner with large empty lumen cells (Figure. 5c). Two layers of fibers in *Danthoniopsis barbata* were less distinctive compared with those in the other two species. A close up of the inner layer of large cells (Figures. 3-5d) revealed plywood or spool-like packing of cellulose fibrils. Antrorse barbs on the surface of diaspore in the three species showed high frequency (Table 1) of 400-450 barbs/mm^2, and a length of 60-70µm. All barbs had a conical shape, with cone-apex angle (δ) of 16-24° (Figure. 6).

DISCUSSION

Drilling effect of trypanospermic diaspores depends mainly on structure and characteristics of twisted part of the awn. Microstructure of this hygroscopically active part revealed two distinctive layers in the three studied species. The outer layer consists of small and thick fiber cells with narrow or filled lumen, and the inner layer made up of large and empty lumen cells. The outer layer represents the surface layer that holds the barbs and in contact to soil particles, hence it must be stiff to support the barbs and protect the inner layer. Mechanical properties of the cell wall depend on its fine structure (Rafsanjani et al., 2014; Albersheim et al., 2011). Fiber cell consists mainly of secondary cell wall where the stiff hydrophobic crystalline cellulose microfibrils are embedded in a soft hydrophilic matrix of amorphous cellulose, hemicellulose, structural protein and lignin, in which hemicellulose cross-links cellulose microfibrils via multiple hydrogen bonds (Keegstra, 2010; Albersheim et al., 2011; Rafsanjani et al., 2014). Cross-linking hemicellulosic molecules could both keep the cellulose microfibrils apart from each other and potentially influence the ability of microfibrils to slip past one another (Albersheim et al., 2011). The tilt angle of microfibrils with respect to cell axis known as microfibril angle (MFA) determines the stiffness of the wood cell. As the MFA increases, tensile strength and stiffness quickly decrease

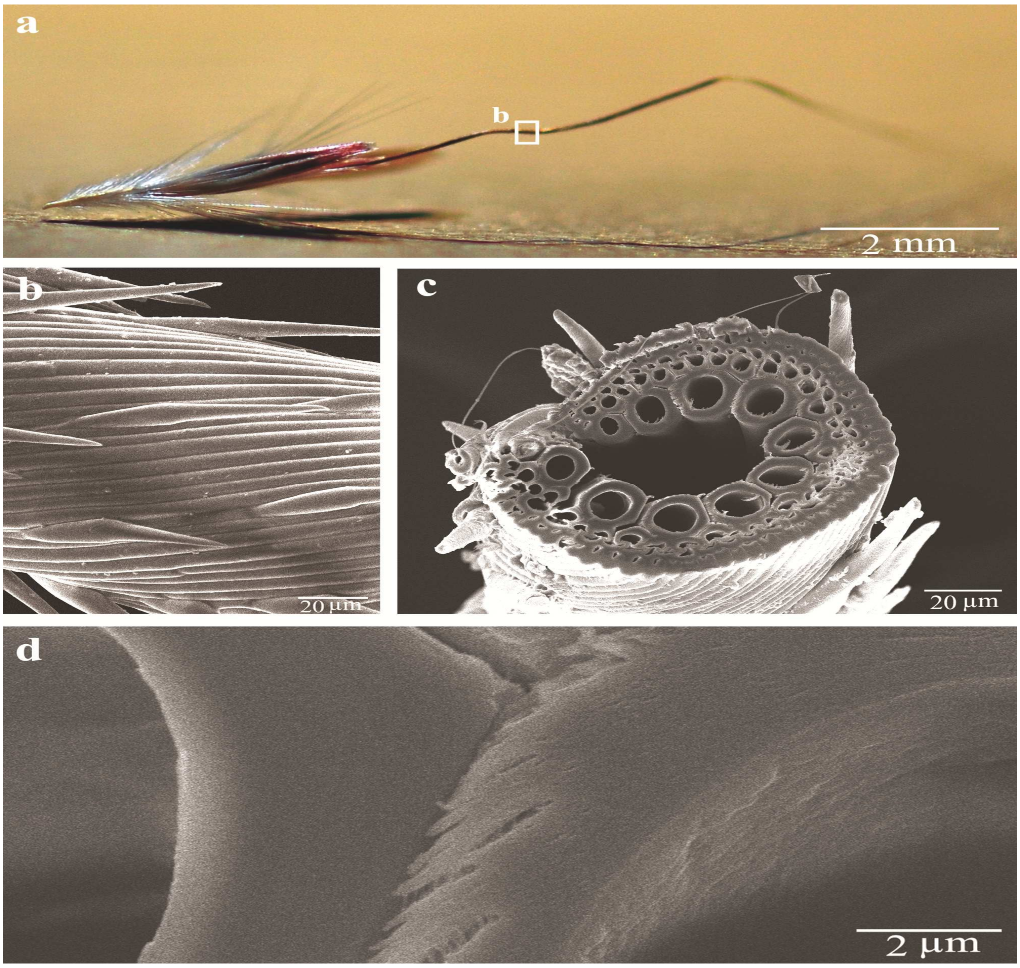

Figure 5. Diaspore of *Dichanthium foveolatum*. **a**: The diaspore, **b**: SEM micrograph of twisted part, **c**: SEM photo micrograph of cross section. **d**: SEM photo micrograph of inner layer revealed spool-like packing of cellulose fibrils in cell wall.

Table. 1. Some characteristics of the barbs in the studied specie

	Aristida funiculata	*Danthoniopsis barbata*	*Dichanthium foveolatum*
Length (µm)	68.8 ± 11.6	70.9 ± 23.5	58.6 ± 12.6
Cone-apex angle	24.2° ± 2.6	19.1° ± 3.9	16.6° ± 4.7
frequency (barb mm^{-2})	428.6 ± 48.8	457 ± 127	400 ± 57.7

(Burgert & Fratzl, 2009; Tabet & Abdul Aziz, 2013). In wood fibers of many species there is an additional layer consisting of almost pure cellulose organized strictly parallel to the cell axis (MFA ~ 0°) that can fill the whole lumen (Burgert & Fratzl, 2009). The outer layer of twisted part (fiber cells with narrow or filled lumen) indicating a small MFA, which reflect the stiffness and strength of fiber cells. In contrast, the inner layer of twisted fiber cells revealed plywood packing of cellulose fibrils in all three species. This structure indicating a big MFA (Elbaum et al., 2007, 2008) which means less stiffness and strength, making fibers highly responsive to humidity changes (Elbaum & Abraham, 2014). The porous soft cell wall matrix enables rapid penetration of water molecules into the matrix causing expansion of the structure and leading to swelling of wet tissues. Since the combination with

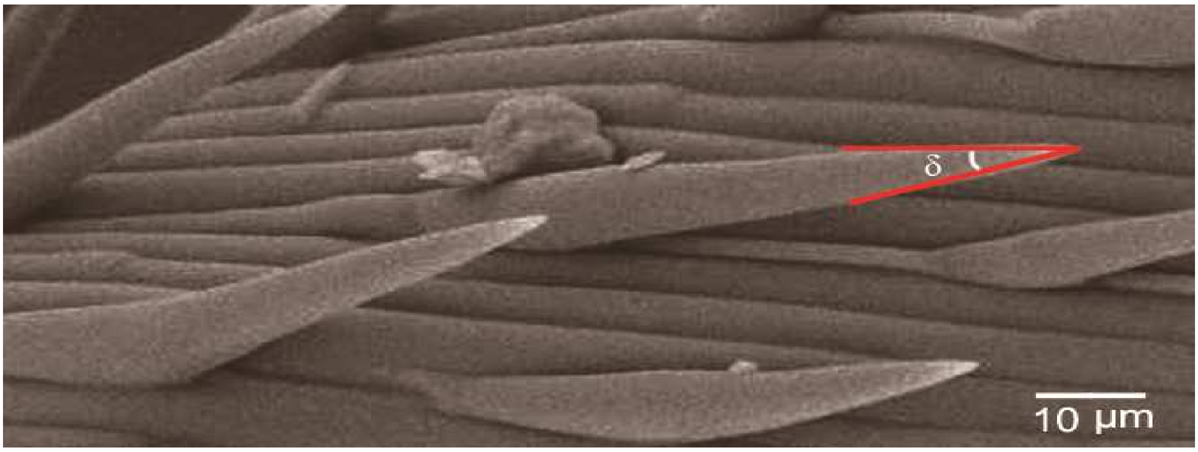

Figure 6. Barbs on twisted part of *Danthoniopsis barbata*. All barbs had a conical shape, with cone-apex angle (δ) = 19.1° ± 3.9.

water molecules is reversible, the swollen tissue can shrink back to its original size when dry (Jung et al., 2014). The cell wall contracts in a direction essentially perpendicular to the plane formed by the microfibrils, so the direction of contraction is determined by MFA (Fratzl et al., 2008). Moreover, an inner layer with big MFA means a tilted helical arrangement of cellulose microfibrils (Abraham et al., 2012), whereas an outer layer with small MFA means that microfibrils are arranged along the long axis of the cell (Burgert & Fratzl, 2009). Absorption of water in such cells leads to expanding and uncoiling of twisted fibers, whereas loss of water leads to shrinkage and coiling of fibers. These changes enable awns to twist as they dry out and straighten as they become wet, consequently moving the dispersal units along the soil surface until reaching an optimum favorable position for drilling with the pointed callus (usually cracks in soil surface). This mechanism of twisting and untwisting of hygroscopic part enhances the drilling action when the diaspore encounters loose soil particles or cracks on soil surface.

Furthermore, diaspores of *Aristida funiculata* are larger than those of the other two species, with longer twisted part. This species is common in soil with relatively hard soil crust of Tihama Hill slopes (Masrahi, 2012). The size of the twisted part induce a bigger rotating force (drilling effect) compared to the other two species. This effect enhances establishment of this species in the hard soil crust in its natural habitat. With its longer twisted part, the vascular bundles along the column perhaps assist in water transport to the caryopsis when available in adequate levels. Diaspores of both *Danthoniopsis barbata* and *Dichanthium foveolatum* inhabited areas with more loose soils in mountains and coastal area, respectively. Therefore, dimensions of diaspores in these two species are suitable for these types of soil. Barbs on the outer surface of twisted part in all three species are backward direction, which enhances an anchoring effect, leading to effective penetration of callus into soil without pushing the caryopsis out of the ground. This characteristic enhances establishment of the diaspore in a suitable microsite for germination. Additionally, barbs on the twisted part of the awn are conical. This conical shape is known to generate a Laplace pressure gradient (Lorenceau & Quéré, 2004) due to curvature radii, from the high-curvature region (tip of the cone) to the low-curvature region (base of the cone). Any water drop deposited on such a conical shape will move spontaneously towards the region of lower curvature (base of the cone). The tip of the barb (small radius-high curvature region) has a larger Laplace pressure than the base of the barb (large radius-low curvature region). Energy generated from the pressure difference drives the droplet to move from the tip to the base of the barb (Azad et al., 2015; Chen & Zheng, 2014; Sun et al., 2014). The cone-apex angle of the barb must be within a range of limited degree. Angles smaller than 6° will be too small to generate enough Laplace pressure gradient (Ma et al., 2015). An angle close to 45° is too large to be a cone-apex and, consequently too big to generate Laplace pressure gradient. Hence, the barbs on the hygroscopic part of the three species are geometrically ideal to generate a Laplace pressure difference that acts on the water droplet which directly moves to the base where it is absorbed by the highly hygroscopic part. Distribution of silicified barbs, with such a geometrically ideal conical shape that generates a gradient in Laplace pressure, enhances moisture collection leading to activation of hygroscopic movement and drilling effect.

Furthermore, in surface energy gradient principle (Brochard, 1989; Chaudhury & Whitesides, 1992) a water drop tends to be driven towards the more wettable region, with higher surface energy. This effect results in movement of the water droplet on the barb from the less wettable silicified barb with low surface energy to the wettable hygroscopic twisted high surface energy fibers. Once the water droplet contacts this hygroscopic highly hydrophilic surface, it will be rapidly absorbed. Under atmospheric humidity conditions, the spontaneous droplet transportation makes it possible for a continuous water

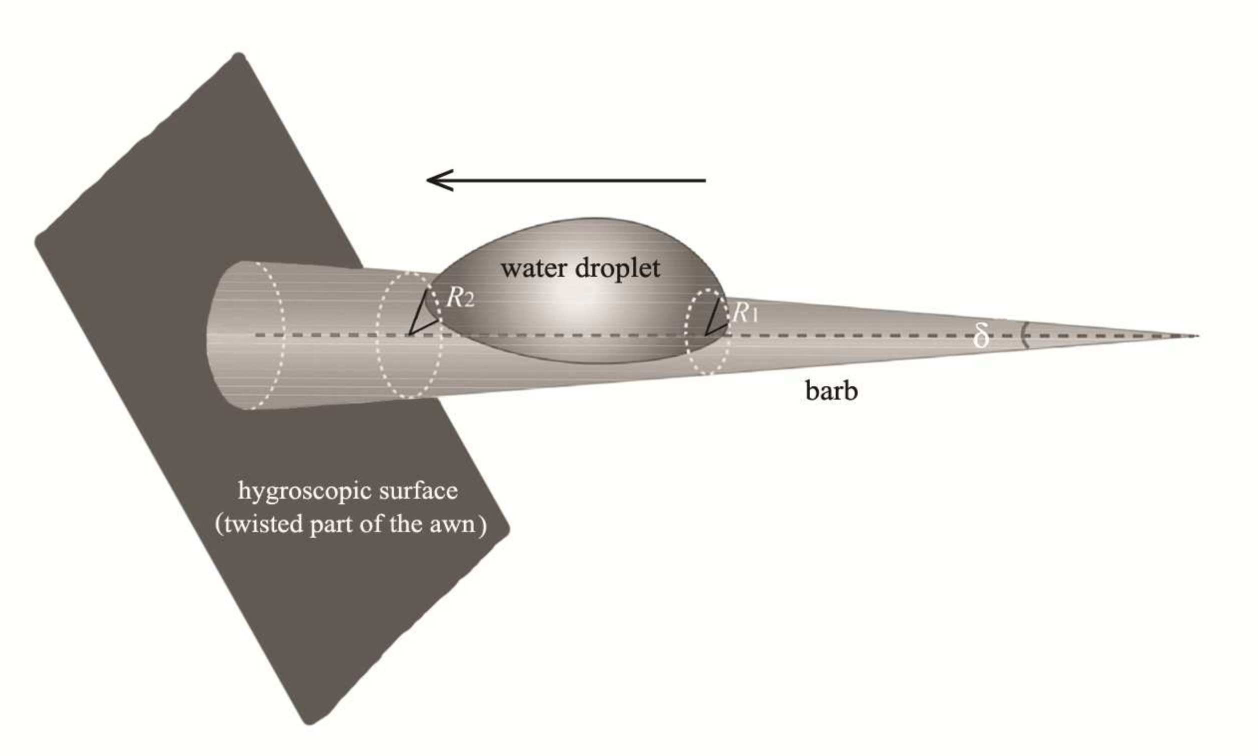

Figure 7. The two forces that drive water droplets from the tip of the barb to the base. 1- Laplace pressure gradient, in which the water drop tends to move from the high-curvature region (tip of the cone, small radius) to the low-curvature region (base of the cone, large radius). 2- Surface energy gradient, in which the water drop tends to be driven towards the more wettable region (hygroscopic surface).

droplet collection by releasing the fresh barb surface. Water droplet is preferentially captured by the tip site in the cone structure (Cao et al., 2014). These two forces drive water droplets from the tip of the barb to the base (Figure. 7) even if the barb array is turned upside down as in lower surface of the twisted part when it is lying down on the soil.

The high frequency of barbs on outer surface of the twisted part enhances moisture collection. In *Danthoniopsis barbata* the barbs are found on both outer and inner surfaces, with large cleavage on the inner surface. This perhaps make it more efficient in moisture collection.

High humidity of the coastal area in Jazan region and large diurnal temperature changes cause the air to become supersaturated with moisture during the cool night and precipitation in the form of dew occurs on suitable surfaces (MEPA, 1992). This phenomenon is most significant in winter months where the nights are cooler. The same phenomenon occurs in the mountains when the temperature falls below dew point.

All three species are annuals that germinate after summer rain in the region (Masrahi, 2012). As the winter approaches the diaspores are in full maturity and fallen on the ground around the mother plants. At this time the diaspores are ready as hygroscopic devices. These climatic conditions enhance water droplet collection by the barbs. The features of the barbs and highly sensitive twisted fibers are characteristics that support hygroscopic movements leading to well establishment of the diaspores in suitable soil microsite for germination.

CONCLUSIONS

Microstructure of hygroscopic awns in diaspores of the three species, *Aristida funiculata*, *Danthoniopsis barbata* and *Dichanthium foveolatum* exhibit very active functional devices. These functional traits include hygroscopically active twisted fiber cells, fine barbs on the surface with geometrically ideal conical shape, and different wettability between barbs and hygroscopic surface of twisted fibers. These characteristics ensure moisture collection to drilling movement into the soil for diaspores establishment in suitable microsite for germination.

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