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Salt Glands in the Poaceae Family and Their Relationship to Salinity Tolerance

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Abstract The Poaceae is one of the most important Angiosperm families, in terms of morphological diversity, ecology and economic importance. Species within this family show a very wide variation in terms of salinity tolerance. Salt secretion through salt glands plays a significant role in regulating ion balance, contributing to salinity tolerance. This review focuses on salt glands in the Poaceae family and their role in the salinity tolerance. In Poaceae microhairs have been observed in all subfamilies, except Pooideae, but functioning salt glands are reported only in genera belonging to the Chloridoideae subfamily. Structural, ultrastructural and physiological features of salt glands are summarized and discussed and the use of salt glands as potential target features for improving salt tolerance of crops is considered.

Keywords Salt gland · Poaceae · Microhairs · Salinity tolerance

Introduction

Soil salinity is an important abiotic stress factor that affects crop productivity all over the world (Ashraf, 1999; Borsani et al., 2003; Chinnusamy et al., 2005; Yadav et al., 2012; Brini & Masmoudi, 2012; Jouyban, 2012). Globally, it has been estimated that more than 800 million hectares of land are affected by salt (Munns & Tester, 2008).

Saline soils are characterized by a high concentration of various soluble salts (Tester & Davenport, 2003), which impose both water-stress and ion-specific limitations that in turn can result in ion imbalances and plant toxicitys, as outlined in the pioneering

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review by (Greenway & Munns, 1980). Sodium and chlorine salts are commonly associated with salinity conditions, with most plants being sensitive to excess concentrations of those ions (Tester & Davenport, 2003). Plant adaptation to saline conditions include mechanisms that contribute to access restriction of these and other potentially deleterious ions to metabolically active sites, both at organ (Zhang & Blumwald, 2001; Davenport et al., 2005) and subcellular levels (Sottosanto et al. 2004; Tester & Davenport, 2003), osmotic balance provided by organic or inorganic molecules (Zhang et al., 1999) and reactive oxygen species detoxifying mechanisms (Mittova et al., 2002; Buchanan et al., 2005; Taleisnik et al., 2009)

In some species, excess ions are secreted through structures that have evolved on the surface of the aerial parts (Thomson & Healey, 1984). Salt secretion (also referred to as recretion or excretion) through salt glands plays a significant role in reducing ion concentration in shoots (Waisel, 1972). Saline solutions crystallize above the cuticle and crystals are either blown away or washed off by rain, thus providing an efficient mechanism for removing ions from shoots.

This review focuses on salt glands in the Poaceae family. Structural, ultrastructural and physiological features are summarized and discussed and the use of salt glands as potential target features for improving salt tolerance of crops is discussed.

Salt Gland Occurrence and Evolution in Plants

Halophytic species, which are adapted to grow in highly saline areas, represent about 1 % of the world's flora (Flowers & Colmer, 2008). They can be grouped into three types according to morphological features, ecological behavior and physiological mechanism of tolerance: euhalophytes, pseuhalophytes and recretohalophytes (Breckle, 1995). The latter are characterized by structures that can either excrete salt (salt glands; exo-recretohalophytes) or sequester it (salt bladders; endo-recretohalophytes) and thus remove excess salt from metabolically active tissues (Zhou et al., 2001). In halophytes, these structures play an important role in regulating ion balance, contributing to salinity tolerance (Zhang et al., 2003). Yet, salt glands are not exclusive to halophytes. In *Spartina* Schreb., for example, salt glands occur in both salt marsh and freshwater species, indicating that they may be an ancestral trait in this genus (Flowers et al., 2010).

The evolution of salt glands is uncertain, and it is unclear whether salt glands evolved from glands that originally performed some other function (Ramadan & Flowers, 2004). As they are found in halophytic species that are not closely related taxonomically, convergent evolution of a common adaptive feature has been suggested (Fahn, 1979; Wahit, 2003).

Three types of glands have been described: the bladder cells of the Chenopodiaceae; the multicellular glands observed in dicotyledonous species of the families Acanthaceae, Aizoaceae, Aveceniaceae, Combretaceae; Convolvulaceae; Frankiaceae, Plumbaginaceae and Tamaricaceae (Waisel, 1972; Wahit, 2003; Kobayashi, 2008; Flowers et al., 2010); and the bicellular glands found in species of the Poaceae family (Liphschitz & Waisel, 1974; Fahn, 1979; Wieneke et al., 1987; Mauseth, 1988; Thomson, 1975; Marcum & Murdoch, 1994; Somaru et al., 2002; Wahit, 2003). In addition, unicellular hairs with salt secretion ability have been observed in some

Poaceae, such as *Porteresia coarctata* (Roxb.) Tateoka and *Oyiza sativa* L. (Bal & Dutt, 1986; Flowers et al., 1990; Balakrishna, 1995; Latha et al., 2004; Kobayashi, 2008). Bicellular and unicellular glands co-exist in the same leaf in *P. coarctata* and *O. sativa*. However, in *O. sativa* the bicellular glands seem to have low ability to secrete ions, due to the absence of partition membranes (Amarasinghe & Watson, 1988).

Salt Glands in the Poaceae Family

The Poaceae is one of the most important families among angiosperms in terms of morphological diversity, ecology and economic importance (Clayton & Renvoize, 1986; Grass Phylogeny Working Group, 2001; 2012). It includes about 10,000 species and over 700 genera spread all over the world (Tzvelev, 1989; Renvoize & Clayton, 1992; Watson & Dallwitz, 1992; Jacobs et al., 1999; Grass Phylogeny Working Group, 2001; 2012). Species within this family show a very wide variation in terms of salinity tolerance (Marcum, 2008).

Salt glands in grasses were first mentioned as such in the halophytic genus *Spartina* (Skelding & Winterbotham, 1939), but they had been previously described as hydathodes that secreted salt by Sutherland and Eastwood (1916). Microhairs have been observed in all grass subfamilies, except Pooideae (Liphschitz & Waisel, 1982; Amarasinghe & Watson, 1988; 1989; Kobayashi, 2008), but functioning salt glands are reported only in genera belonging to the Chloridoideae subfamily (Amarasinghe & Watson, 1988; 1989; Liphschitz & Waisel, 1974; Taleisnik & Anton, 1988; Marcum et al., 1998; Ramadan, 2001; Bell & O'Leary, 2003; Chen et al., 2003; Wahit, 2003; Koyro & Huchzermeyer, 2004; Marcum & Pessarakli, 2006; Kobayashi et al., 2007; Hameed et al., 2013).

Within Chloridoideae, salinity tolerance has been associated with excess ion exclusion, accompanied in some cases by ion secretion from leaf salt gland microhairs (Figs. 1e–f, 2 and 3c), and with accumulation of compatible solutes such as glycine betaine and proline (Marcum & Murdoch, 1994; Marcum, 1999). Chloridoideae is considered to be a specialized group in stressful environments (Clayton & Renvoize, 1986; Columbus et al., 2007; Peterson et al., 2010) and the occurrence of salt glands in this subfamily would support this role (Taleisnik & Anton, 1988).

Anatomical and Functional Features of Salt-Secreting Microhairs

Microhairs in grasses (Figs. 1–3) are small, bicellular structures, ranging from 15 to 70 μm (Marcum et al., 1998; Marcum & Murdoch, 1990; Marcum, 1999; 2008), with relatively thin walls (Metcalfe, 1960); they can be distinguished from macrohairs, which are large, thick-walled, unicellular trichomes. There are also tricellular microhairs in *Chloris gayana* Kunth (Waisel, 1972; Flowers et al., 1990; Ramadan & Flowers, 2004). These trichomes are termed microhairs by anatomists and salt glands by physiologists (Skelding & Winterbotham, 1939; Liphschitz & Waisel, 1974; 1982; Oross & Thomson, 1982a). Microhairs are found in leaf blades (Tateoka et al., 1959; Metcalfe, 1960; Somaru et al., 2002; Tivano, 2011), leaf sheaths (Somaru et al., 2002;

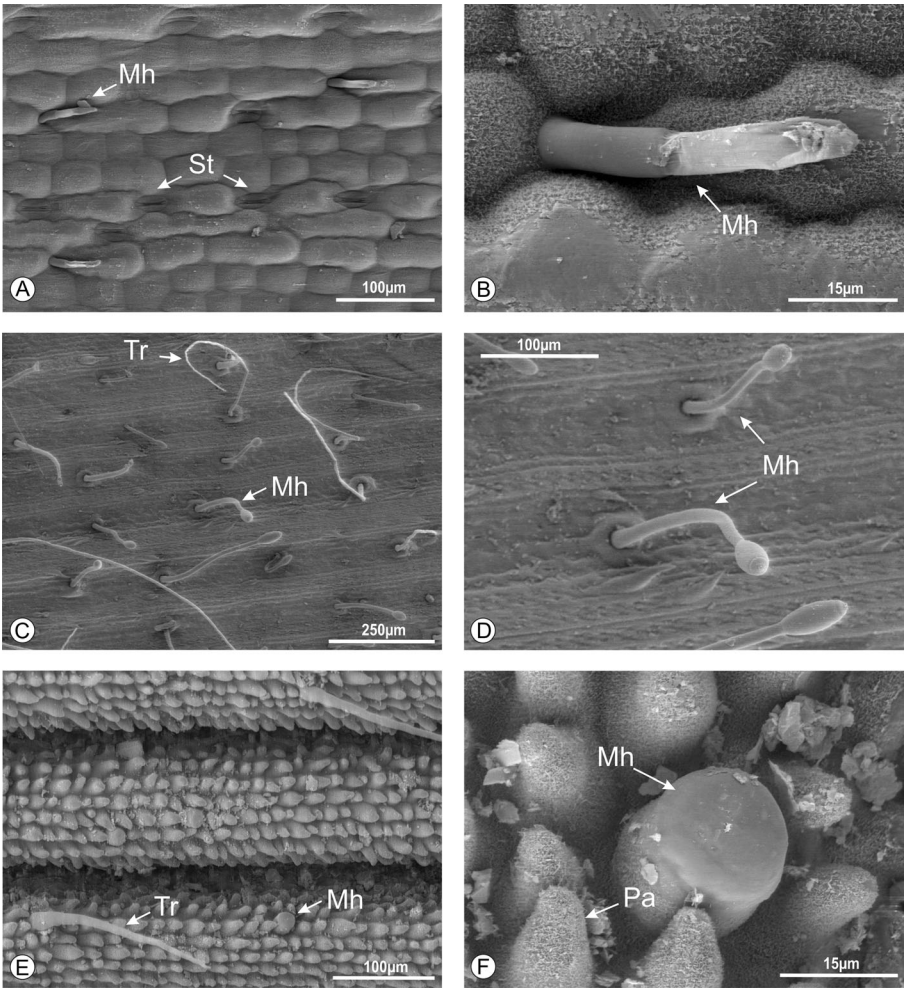

Fig. 1 **a**–**b**, abaxial leaf epidermis (**a**) and microhair "Panicoid type" (**b**) of *Sorghum halepense* (L.) Pers*;* **c**–**d**, abaxial leaf epidermis (**c**) and microhair "Enneapogon type" (**d**) of *Enneapogon desvauxii* P. Beauv.*;* **e**–**f**, adaxial leaf epidermis (**e**) and salt gland microhair the "Chloridoid type" (**f**) of *Distichlis humilis* Phil. **References**: *Pa* papillae, *Tr* trichome, *Mh* microhair, *St* stoma

Tivano, 2011), lemmas, paleas and lodicules (Tateoka & Takaji, 1967; Tateoka, 1976; Scholz, 1979; Terrel & Wergin, 1981; Liu et al., 2010; Tivano, 2011), culms (Arriaga, 1992; Tivano, 2011), inflorescence peduncles and inflorescence rachises (Tivano, 2011).

In leaves, salt glands are distributed in intercostal rows, between rows of stomates (Fig. 2a–e), and are normally found on both leaf surfaces (Liu et al., 2006; Barhoumi et al., 2008). The number of salt glands per unit leaf area was reported to be equivalent on the adaxial and abaxial leaf epidermis in *Aeluropus littoralis* (Gouan) Parl., *Aeluropus lagopoides* (L.) Trin. ex Thwaites and *Ochthochloa compressa* (Forssk.) Hilu (Liu et al., 2006; Barhoumi et al. 2008). In some species, gland density may differ between leaf surfaces. In the adaxial surface, it is approximately three times higher in *Pappophorum philippianum* Parodi than in *Pappophorum pappiferum* (Lam.) Kuntze

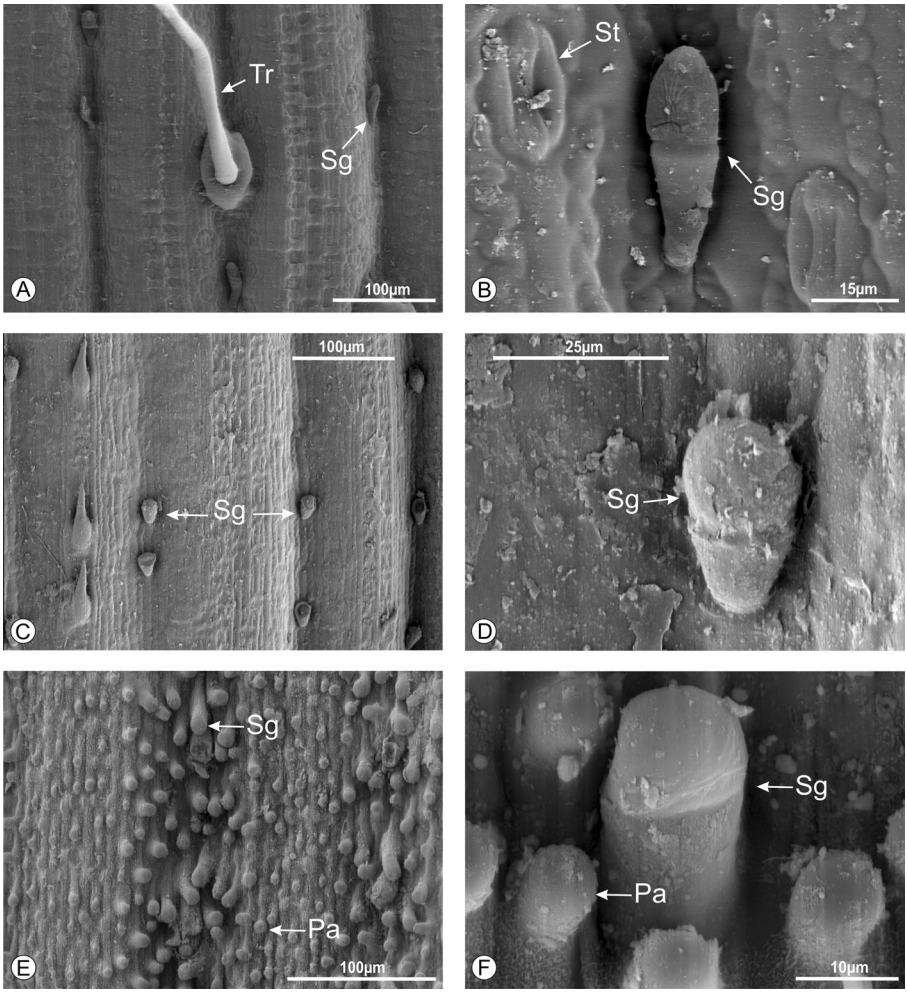

Fig. 2 Some examples of salt gland microhair ("Chloridoid type microhair"): **a**–**b**, abaxial leaf epidermis (**a**) and salt gland microhair (**b**) of *Bouteloua aristidoides* (Kunth) Griseb*;* **c**–**d**, abaxial leaf epidermis (**c**) and salt gland microhair (**d**) of *Munroa argentina* Griseb.*;* E-F, abaxial leaf epidermis (**e**) and salt gland microhair (**f**) of *Diplachne fusca* (L.) P. Beauv. ex Roem. & Schult . **References**: *Pa* papillae, *Tr* trichome, *Sg* salt gland microhair, *St* stoma

(Taleisnik & Anton, 1988); on the abaxial surface, however, gland density is similar in both species. Density may increase in response to salt concentration in the substrate (Naz et al., 2009).

Salt gland basic structure is similar in all genera (Kobayashi, 2008). Each bicellular microhair is composed of a basal cell and a cap cell, attached to or embedded in the leaf epidermis (Levering & Thomson, 1971; Oross & Thomson, 1982a; Naidoo & Naidoo, 1998; Somaru et al., 2002; Barhoumi et al., 2008). The basal cell is the collecting cell whereas the upper cell is the excreting one (Liphschitz & Waisel, 1982). The cap cell commonly protrudes from the leaf surface and the basal cell is embedded in the epidermal cells, with its base in contact with the mesophyll cells (Barhoumi et al., 2008).

Salt glands appear individually (Figs. 2–3), except in *Zoysia tenuifolia* Thiele, where they are clustered into groups of two or three (Marcum et al., 1998). They can be surrounded by papillae, as in the adaxial epidermis of *A. littoralis* (Marcum et al., 1998; Marcum, 2008). In the abaxial epidermis of this species, salt glands are protected by trichomes (Barhoumi et al., 2008). In *Odyssea paucinervis* (Nees) Stapf, each gland is protected by four epidermal trichomes; the salt gland and these four trichomes form the salt gland complex (Somaru et al., 2002). Salt gland microhairs may be located more or less deeply in the epidermis (*Distichlis humilis* Phil. (Fig. 1e–f), *Spartina*), with the basal cell semi-embedded (*Diplachne fusca* (L.) P. Beauv. ex Roem. & Schult. (Fig. 2e–f), *Cynodon* Rich., *Tetrapogon* Desf.) or arranged above the epidermal cells (*Bouteloua aristidoides* (Kunth) Griseb. (Fig. 2a–b, *Munroa argentina* Griseb. (Fig. 2c–d) and *Z. tenuifolia*) (Liphschitz & Waisel, 1974; Marcum & Murdoch, 1994; Marcum, 1999; Marcum, 2008).

Within a common structural pattern, variations in form, ultrastructure and function of the salt glands have been described (Reeders, 1964; Liphschitz & Waisel, 1982; Amarasinghe & Watson, 1988; Taleisnik & Anton, 1988; Barhoumi et al., 2008). In general, three types of microhairs have been described in Poaceae (Fig. 1): the "Panicoid type" (Fig. 1a–b), the "Enneapogon type" (Figs. 1c–d and 3d) and a third, rare type, the "Chloridoid type" (Figs. 1e–f, 2 and 3a–c). The Chloridoid type is typical of the Chloridoideae subfamily; the Panicoid type appears in the Panicoideae, Arundinoideae and Bambusoideae subfamilies as well as in a few genera of Chloridoideae (Watson et al., 1985; Watson & Dallwitz, 1994). Different species of the genus *Eragrostis* Wolf.; however, may exhibit either the Panicoid or Chloridoid type of glands or intermediate forms between these two types (Tateoka et al., 1959; Amarasinghe & Watson, 1988). The Enneapogon microhair type appears in *Enneapogon* Desv. ex P. Beauv., *Cottea* Kunth., *Kaokochloa* De Winter., *Schmidtia* Steud. ex J.A. Schmidt. (Tateoka et al., 1959; Stewart, 1964; Tivano, 2011) and also in *Amphipogon* R. Br. (Johnston & Watson, 1976; Watson et al., 1985) and *Neeragrostis reptans* (Michx.) Nicora (Renvoize, 1985; Nicora & Rúgolo de Agrasar, 1987). This microhair type is absent in all species of *Pappophorum* Schreb. (Stewart 1964).

Two types of microhairs can be distinguished in the Chloridoids, according to the presence of "partitioning membranes" in the basal cell (as in *Chloris* Sw., *Dactyloctenium* Willd., *Eleusine* Gaertn., *Leptochloa* P. Beauv., *Sporobolus* R. Br. and *Zoysia* Willd.) or their absence (as in *Eragrostis cilianensis* (All.) Vignolo ex Janch., *Eragrostis parviflora* (R. Br.) Trin. and *Pogonarthria squarrosa* (Roem. & Schult.) Pilg.) (Amarasinghe & Watson, 1989; Amarasinghe, 1990). Partitioning membranes are considered to be crucial for the salt secretion processes in Chloridoid grasses (Levering & Thoompson, 1972; Oross & Thomson 1982a; Amarasinghe & Watson, 1988; Barhoumi et al., 2008). Salt secretion was not detected in any of the microhairs lacking basal cell "partitioning membranes", whereas Chloridoid-type microhairs of *Sporobolus elongates* R. Br. and *Eleusine indica* (L.) Gaertn. were not seen to secrete salt, despite the presence of partitioning membranes (Amarasinghe & Watson, 1989; Amarasinghe, 1990).

Taleisnik & Anton (1988) described salt glands in two species of *Pappophorum* (*P. philippianum* and *P. pappiferum*). The microhairs present in *Pappophorum* are Chloridoid type (Cáceres, 1958; Renvoize, 1985). The bicellular hairs of *Pappophorum* (Fig. 3c) are somewhat different from those found in other members of the

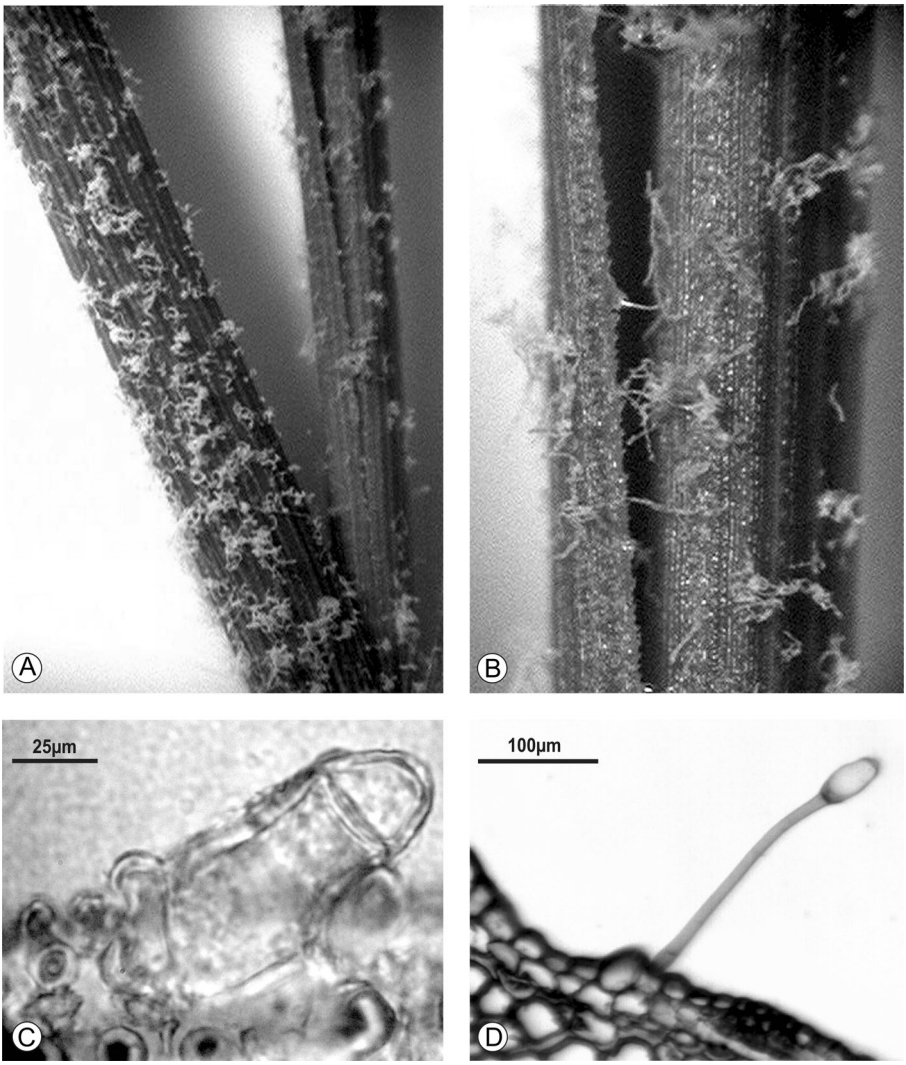

Fig. 3 **a**–**b**, salt excretions in leaf (**a**) and in culm (**b**) of *Pappophorum philippianum* Parodi; **c**, salt gland microhair in leaf adaxial epidermis of *P. philippianum*; **d**, "Enneapogon" microhair type in culm of *Schmidtia kalihariensis* Stent

Pappophoreae (Figs. 1c, d and 3d) (Reeder, 1964). On the basis of these and other characters, this author proposed to locate *Pappophorum* in a different subtribe from the other genera of the *Pappophoreae* tribe. It has been widely accepted that the tribe Pappophoreae s.l. is polyphyletic (Columbus et al., 2007; Reutemann et al., 2011). Peterson et al. (2010) proposed the division of this tribe into two subtribes: Cotteinae within Eragrostideae and Pappophorinae within Cynodonteae. The Cotteinae subtribe shows the Enneapogon microhair type (Figs. 1c–d and 3d).

Panicoid type microhairs (Fig. 1a–b) have long, narrow cap cells with a relatively high length/width ratio. The Chloridoid type has a hemispherical cap cell with a relatively low length/width ratio (Figs. 1e–f and 2 c). Tateoka et al. (1959) characterize

the Enneapogon microhair type (Figs. 1c–d and 3d) as having a delicate basal cell with highly varying length and an oblong cap cell of constant length.

Partitioning membranes are an intricate membrane system in the cytoplasm, being the most prominent features of salt gland basal cells (Oross & Thomson 1982a). These irregularly shaped structures are more or less elongated and show no defined orientation (Levering & Thomson, 1971; Oross & Thomson, 1982a; b; 1984; Oross et al., 1985; Naidoo & Naidoo, 1998; Somaru et al., 2002; Barhoumi et al., 2008). They form open channels in the direction of ion flow (Amarasinghe & Watson, 1988). Oross & Thomson (1982b) suggested that they are extensive invaginations of the plasmalemma, so the space between them is actually apoplastic. These authors described an apoplastic continuum between the leaf mesophyll cells and a system of membranous extracellular channels, suggesting that this continuum may function in the absorption of solutes from the apoplast (Oross & Thomson, 1982a; Amarasinghe & Watson, 1988). In *Spartina*, partitioning membranes extend from wall protuberances that project into the basal cell from the wall between the cap and basal cell (Levering & Thomson, 1971), but such protuberances are absent in *Cynodon*, *Distichlis* Raf. and *A. littoralis* salt glands (Oross and Thomson 1982a; Barhoumi et al., 2008). Partitioning membranes are usually observed in close association with microtubules and resemble endoplasmis reticulum. This led Barhoumi et al. (2008) to hypothesize that partitioning membranes may be modified endoplasmic reticulum rather than an infolding of the plasmalemma, as previously suggested. The basal cell of the Chloridoid microhairs with partitioning membranes has few vacuoles, a large nucleus, a relatively dense cytoplasm with a rough endoplasmic reticulum, free ribosomes and numerous mitochondria. Microtubules usually run in parallel with the "partitioning membranes". Compared with basal cells having partitioning membranes, the basal cells of Chloridoid microhairs that lack partitioning membranes show a prominent nucleus but relatively few mitochondria (Amarasinghe & Watson, 1988).

Plasmodesmata are not detected between the basal cell and the neighboring epidermal cells; however, numerous plasmodesmata occur in the common walls between the basal and mesophyll cells in *Spartina foliosa* Trin. (Levering & Thomson, 1971) and in *A. littoralis* (Barhoumi et al., 2008). These plasmodesmata are located in restricted and relatively thin zones of the common walls, termed the "transfusion area" (Barhoumi et al., 2008). In *Zoysia matrella* (L.) Merr. cultivar 'Cavalier', a symplastic connection is observed between the salt gland and the neighboring epidermal cells, suggesting a role for the epidermal cells as a reservoir for salt storage before it is transported to the salt glands (Rao, 2011).

As in epidermal cells, salt gland microhairs have cutinized cell walls; the basal cell wall is thicker and more cutinized than the cap cell (Taleisnik & Anton, 1988). The cuticle overlying the microhair is continuous with the adjoining epidermal cells. This cuticle does not fully cover the basal cell (Amarasinghe & Watson, 1988). No cuticular layer was observed between the mesophyll and the basal cell (Levering & Thomson, 1971; Amarasinghe & Watson, 1988). The portion of the cuticle above the cap cell is thicker than that along the sides (Amarasinghe & Watson, 1988). In some genera, the cuticle in the cap cell has numerous pores. In *D. fusca*, each gland is provided with a centrally located pore (Joshi et al., 1983) through which salt may be secreted.

In the distal end of the microhair, the cuticle is always detached from the cap cell wall, forming a large chamber. This chamber has been observed mainly in Chloridoid-

type and Enneapogon-type microhairs (Levering & Thomson, 1971; Oross & Thomson, 1982a; Amarasinghe & Watson, 1988; Naidoo & Naidoo, 1998; Somaru et al., 2002; Barhoumi et al., 2008). In salt secretory microhairs, the cuticular chamber functions as a collecting compartment in which salt accumulates before being secreted via the cuticle (Campbell & Thomson, 1976; Fahn, 1979; Amarasinghe & Watson, 1988). In *A. littoralis*, the collecting chamber is covered by a cuticle about 130 nm thick. Above the protruding portion of the cap cell cuticle, there is an electron-dense layer that is about one half the thickness of the cuticle, which has been suggested to play a protective role (Barhoumi et al., 2008).

Pathway of Ion Transport and Secretion

Ion transport pathways to the basal cell can be apoplastic (Oross & Thomson, 1982b; Oross et al., 1985; Naidoo & Naidoo, 2006) or symplastic (Kobayashi, 2008). The combination of apoplastic and symplastic pathways has also been suggested (Naidoo & Naidoo, 1999, 2006). The apoplastic movement is facilitated by the absence of cutin in the walls between the mesophyll cells and the basal cell of the gland (Levering & Thomson, 1971; Oross & Thomson, 1982a; Naidoo & Naidoo, 1999). Plasmodesmata in the transfusion area between the mesophyll cells and the basal cell may be part of a symplastic ion transport pathway (Levering & Thomson, 1971; Naidoo & Naidoo, 1999; Wahit, 2003; Barhoumi et al., 2008; Kobayashi, 2008). Ions are suggested to move symplastically from the basal to the cap cell, through the abundant plasmodesmata connecting them (Pollak & Waisel, 1970; Levering & Thomson, 1971; Barhoumi et al., 2008). This type of transport is not observed between adjoining epidermal cells and the basal cell, because they are not connected by plasmodesmata (Barhoumi et al., 2008).

Salt accumulation occurs in amorphous vacuoles in the basal and cap cells (Thomson & Liu, 1967; Thomson et al., 1969; Somaru et al., 2002; Barhoumi et al., 2008). These small vacuoles may fuse with the plasmalemma of the cap cell and release their content into the cuticular chamber prior to secretion (Naidoo & Naidoo, 1999; Wahit, 2003). Solutions accumulate in the cuticular chamber and then either they are secreted through the pores in the cuticle or the cuticle may eventually break, releasing the solution on the leaf surface (Naidoo & Naidoo, 1999; Levering & Thomson, 1971). In leaves of *C. gayana*, microhairs excrete salt continuously through the wax-free cuticle of the cap cell without rupturing the cuticlular structure (Oi et al., 2013a, 2014).

Composition of Secreted Salts

Salt glands of Poaceae secrete a wide variety of ions (Kobayashi, 2008). The type and concentration of secreted ions may vary according to the ion composition of the substrate (Oi et al., 2013b). Salt glands can secrete Na^+, K^+, Ca^+, Mg^+, Cl^- (Thomson, 1975; Liu et al., 2006; Oi et al., 2013b). Salt glands can also secrete some organic substances, such as soluble sugars, amino acids and small proteins (Pollak & Waisel, 1970). Secretion of Na^+ and Cl^- is higher than that of other ions (Arisz et al., 1955; Scholander, 1968; Pollak & Waisel, 1970; Joshi et al., 1983; Somaru

et al., 2002; Liu et al., 2006; Kobayashi, 2008; Marcum, 2008). The secretion mechanism has a low affinity toward the divalent cations Ca^{+} and Mg^{+} (Pollak & Waisel, 1970; Rozema et al., 1981; Joshi et al., 1983; Wieneke et al., 1987; Ramadan, 2001; Marcum, 2008). The secretion of SO_4 is scarce and NO_3 secretion has been observed only in a few species (Klagges et al., 1993; Kobayashi & Masaoka, 2008). A low amount of PO_4 was been detected in the secretions of *Spartina alterniflora* Loisel. and *Aeluropus pungens* (M. Bieb.) K. Koch (McGovern et al., 1979; Chen et al., 2003).

Some species (*A. lagopoides*) tend to secrete potentially toxic ions and retain physiologically beneficial ions like Ca^{+} and K^{+}, whereas other species (*O. compressa*) excrete all ions, without discrimination between toxic or beneficial (Naz et al., 2009). The salt glands of Rhodes grass can secrete both Na^{+} and K^{+}, but Na^{+} secretion is higher (Kobayashi et al., 2007). The application of various ion transport inhibitors to detached leaves suggested different secretion mechanisms for Na^{+} and K^{+} (Kobayashi et al., 2007).

Metal ions, such a Fe^{+}, Se^{+}, Fe^{+}, Mn^{+}, Zn^{+}, Cd^{+}, Cr^{+}, Cu^{+}, Hg^{+}, Ni^{+} and Pb^{+}, were detected in the secretions of some species of Poaceae (Krauss et al., 1986; Krauss, 1988; Wu et al., 1997; Burke et al., 2000; Windham et al., 2001; Chen et al., 2003; Kobayashi, 2008). Some metals taken up by plants can be released back to the marsh systems through the secretion from salt glands (Krauss et al. 1986; Krauss, 1988), as reported in the marsh communities of *S. alterniflora* (Burke et al., 2000). However, secretion rates observed by these authors are far higher than those reported by Krauss et al. (1986) and Krauss (1988).

Salt Gland Secretion Mechanisms

In grasses having active glands, salt crystals can be observed on leaves of plants growing in soils with high salt concentration (Liphschitz & Waisel, 1974; Taleisnik & Anton, 1988; Tivano, 2011). These crystals are an evidence of salt secretion. For instance, salt crystals were quite evident on leaf surfaces of the facultative halophyte *P. philippianum* in plants grown under saline conditions (Taleisnik & Anton, 1988; Tivano, 2011, Fig. 3a, b), but only a scant secretion was observed in *P. pappiferum*, a glycophyte (Taleisnik & Anton, 1988).

Several hypotheses for salt gland secretion have been proposed, but up to now the mechanisms involved are still not clear (Shabala, 2013). Ions concentrated in vacuoles may be secreted by exocytosis (Ziegler & Lüttge, 1967; Echeverría, 2000). An exocyst protein complex is required for the fusion of the vacuoles to the plasma membrane (Munson & Novick, 2006). The exocyst is involved in the exocytosis of different secretion types (Zhang et al., 2010); however, it is not clear whether it is involved in the mechanism of salt gland secretion in grasses (Ding et al., 2010). These authors propose a model of salt gland secretion in which a vesicle system and membrane-bound transporting proteins are involved. The vesicles may fuse with the plasmalemma and thus salts are excreted, or they may dock onto the plasma membrane without fusion but channels on both membranes would connect them and allow ion secretion to the surface.

Membrane transport proteins play an important role in various processes of salinity tolerance (Bluwald, 2000; Flowers & Colmer, 2008), including the secretion process in salt glands. Almost two decades of extensive molecular studies have clearly established

the involvement of NHX, SOS1, and HKT transporters in plant salt tolerance. Although *HKT*, *SOS1* and *NHX* transporters have been studied and characterized in several different plant species, until recently no attempts had been made to associate the role of ion transporters with salt gland function. Indeed, the work of Rao (2011) presented the first report on the localization of ion transporters in a plant species bearing salt glands, *Z. matrella*. The author worked with cultivars 'Diamond' and 'Cavalier'; he observed different spatial leaf expression patterns for isoforms of *HKT*, *SOS*, and *NHX* in both cultivars and suggested the contribution of those patterns to the specific salt tolerance of these cultivars (Rao, 2011).

Salt Secretion and Gland Density

Increasing salt concentration in the substrate increases secretion rates up to an optimal level and then rates decline (Liphschitz & Waisel, 1982). Salinity levels at which maximum secretion rates are observed vary among species. Maximum rates are observed at between 150 and 200 mM NaCl (8–13 dSm-1) in moderately tolerant Chloridoid species, such as *Cynodon*, *Ch. gayana* and *Eleusine* (Wieneke et al. 1987; Liphschitz & Waisel, 1974; Worku & Chapman, 1998); at 200 mM NaCl (17 dSm^{-1}) in *Distichlis* and *Spartina* (Liphschitz & Waisel, 1974) and at 300 mM NaCl (23 dSm^{-1}) in *Sporobolus* (Marcum & Murdoch, 1992).

There is no agreement among authors about the time of the day of highest salt secretion. Hansen et al. (1976) reported that salt secretion in *Distichlis spicata* (L.) Greene is higher at night. Ramadan (2001) and Marcum (2008) found that salt secretion increased during the night, and contributed to remove salt buildup that occurred during the day. However, Ramadan (1998) reported that more than 67 % of the absorbed salt was secreted by leaves during the day in *Reaumuria hirtella* Jaub. et Sp. The diurnal or nocturnal patterns of salt secretion may possibly be regulated by still unclear environmental factors. Pollak & Waisel (1979) suggested that prevailing high air humidity and the decrease of water stress may be advantageous for night secretion.

Salt secretion and salt gland density can be controlled by plant hormones and ion transport inhibitors (Kobayashi, 2008). The application of ABA appears to affect the Na-secretion process (Wieneke et al., 1987; Kobayashi, 2008). Treatments with cytokinins increased the number of salt glands in some grass species (Liphschitz & Waisel, 1974; Ramadan and Flowers, 2004). Benzyl adenine (BA) increased secretion through its influence on the number of microhairs and leaf area, rather than by affecting the efficiency of the secretion process per se (Ramadan & Flowers, 2004). Kobayashi et al. (2010) showed that exogenous methyl jasmonate (MeJA) alters the density of macrohairs and salt glands in Rhodes grass by reducing leaf area and affecting trichome initiation; macrohair initiation is increased whereas that of salt glands is decreased.

Salt Glands in Salt Tolerance Breeding Programs

Drought and salinity stress are important abiotic factors that limit crop yields (Jiang et al., 2012) and the development of crops that are tolerant to these conditions is a major driver of agricultural research. Specifically, increased crop salt tolerance is a goal for

the productive incorporation of salt-affected soils (Roychoudhury & Chakraborty, 2013). Incorporating traits involved in salt tolerance into crop, woody and fodder plants is a target in conventional and biotechnological breeding schemes (Ashraf & Foolad, 2013). Various physiological and molecular mechanisms associated with plant salt tolerance, including those related to osmoregulation, reactive oxygen species detoxification, ion balance control and signaling events, have been introduced into model and crop plants with the purpose of increasing salt tolerance, as recently reviewed by Reguera et al. (2012)

Can knowledge on the salinity tolerance of salt-gland bearing grasses contribute to increasing salt tolerance in crops? At least two possibilities can be suggested:

1. *Selecting for higher salt gland density.* Salt gland density is an innate, genetically-controlled heritable trait (Marcum, 2008; Rao, 2011). Improved salt tolerance in tetraploid *C. gayana* cultivars (Pérez et al., 2009; Loch & Zorin, 2010) as well as in diploid cultivars (Zorin & Loch, 2007) has been associated with increased salt gland density. Likewise, salinity tolerance was positively correlated with salt secretion and salt gland density in species of *Zoysia* (Marcum et al., 1998; Marcum, 2008; Rao, 2011) and *Sporobolus* (Hameed et al., 2013). Salt glands have been found in wild rice (*Oryza coarctata* Roxb.) (Bal & Dutt, 1986; Yadav et al., 2012) and crosses with this species may be used to increase salt tolerance in *O. sativa*.

   Salt gland density is easily quantified on grass leaves and may be conveniently implemented as a selection tool in breeding programs (Marcum, 2008).
2. *Inducing the development of salt secretion capacity in grass species whose microhairs do not secrete salt*. In maize the number of microhairs per unit area of adaxial leaf surface of the youngest leaf almost doubled as salinity increased from zero to 120 mM NaCl, with a 50 % increase in the number of microhairs on the abaxial surface (Ramadan & Flowers, 2004). Though these microhairs do not secrete salt, microhair density was inducible, and the introduction of salt-secreting capability in microhairs would be challenging. There is at least one instance in which secretion of salt appears to have been induced. McGovern et al. (1995) reported the presence of crystals on the leaves of *Sorghum halepense* (L.) Pers. This species presents Panicoid-type microhairs that do not usually secrete salts. These microhairs could be induced to secrete salt when plants were grown in a soil mixture that was high in lime (McGovern et al., 1995). Inducing salt-secreting capability in microhairs may be complex due to the involvement of anatomical as well as ion-transport features in salt secretion process. The introduction of ion transporters has been successfully used to increase plant salt tolerance (Zhang & Blumwald, 2001), as highlighted in the review on Na homeostasis by Hasegawa (2013). Introducing ion transport capacity in microhairs would require their control by site-specific transcription factors and the support of increased transport capability in mesophyll cells. These challenges may currently seem unattainable; however, technological development may render them possible in the near future. Cereal microhairs do not have glandular function and are not big enough to sequester excess Na^+ continuously (Shabala, 2013). Improving salinity tolerance in cereal crops by introducing salt secretion mechanisms relies on the possibility of changing these two features. There is currently little understanding of the

molecular mechanisms that mediate Na^+ excretion through glands; hence, modifying the number, size and shape of trichomes may be the most practical way to improve Na^+ balance in leaves of grass crops (Shabala, 2013).

Salt glands have been studied for many years and are recognized as an integral part of the complex picture of plant salt tolerance. Further attention to the specific molecular components of the salt-secretion mechanism in salt glands and biotechnological attempts to introduce them into non-secreting microhairs will undoubtedly contribute to an increase in salt tolerance in grass crops.

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