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Ion Secretion via Salt Glands in Poaceae

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ABSTRACT

Salinity is a major constraint to plant growth. To adapt to saline environments, plants develop various mechanisms. One such mechanism is salt secretion via "salt glands". Salt glands are specialized epidermal structures that occur on the shoots, and secrete salts transported into the shoots. Some plant species also release heavy metals via salt glands when they grow on heavy metal-contaminated soils. In addition to their contribution to salinity and heavy metal tolerance, ion secretion via salt glands plays a role in the circulation of elements (soil – plant – environment), and affects crop quality by changing their mineral concentration. The family Poaceae includes many important crops and forage grasses, and almost all Poaceae plants except for the subfamily Pooideae are believed to possess active or inactive salt glands. This paper describes diverse aspects of the phenomenon "ion secretion via salt glands in Poaceae" by focusing on recent studies.

Keywords: glandular trichome, Gramineae, heavy metal, ion excretion, salinity tolerance

CONTENTS

INTRODUCTION

Salinity is a major constraint to plant growth. To adapt to saline environments, plants develop various organs. One such organ is a specialized epidermal structure called a "salt gland", which secretes salts transported into the shoots (**Fig. 1**). Salt secretion via salt glands plays an important role in the regulation of salt concentration in plant tissue (Shirata *et al.* 1989; Flowers *et al.* 1990; Ramadan 2001), and there is a significant positive correlation between salinity tolerance and salt secretion ability within 7 species of Chloridoideae plants (Marcum 1999), 57 zoysiagrass accessions and cultivars (Marcum *et al.* 1998) and 35 *Cynodon* spp. turf cultivars (Marcum and Pessarakli 2006).

Salt glands are known to secrete a wide variety of ions. For example, they secrete metal ions, such as Fe, Hg, Mn and Zn, under these metal-rich conditions (Rozema *et al.* 1991; MacFarlane and Burchett 1999; Windham *et al.* 2001). The metal secretion via salt glands is believed to contribute to metal tolerance in plants by eliminating metals in plant tissue (Neumann *et al.* 1995; MacFarlane and Burchett 1999). In addition, salt glands play a certain role in the circulation of metals, because secreted metals on the leaf surface will be released to the surrounding environment (Burke *et al.* 2000; Windham *et al.* 2001).

The family Poaceae includes many important crop and forage species. An increase in salt concentration of the field is one of the most serious agricultural problems, and the demand for salinity-tolerant crops is increasing. Poaceae plants also play an important role in the ecosytems of estuaries and marshes, which sometimes suffer the intrusion of waste water containing heavy metals (Kraus 1988; Burke *et al.* 2000). Almost all Poaceae plants except for the subfamily Pooideae are believed to possess active or inactive salt glands on their leaves (Amarasinghe and Watson 1988, 1989; Ramadan and Flowers 2004), and ion secretion has been examined in many Poaceae species (**Table 1**). Therefore, information about ion secretion via salt glands will be applicable for various Poaceae plants inhabiting saline and/or heavy metal-contaminated sites.

There are some reviews concerned with salt glands (Liphschitz and Waisel 1982; Fahn 1988; Thomson *et al.* 1988; Gorham 1992; Wahid 2003), but these focus mainly on the secretion of sodium chloride in limited plant species. However, the targets of recent studies have expanded to various ions, plant species and roles other than NaCl toler-

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Invited Review

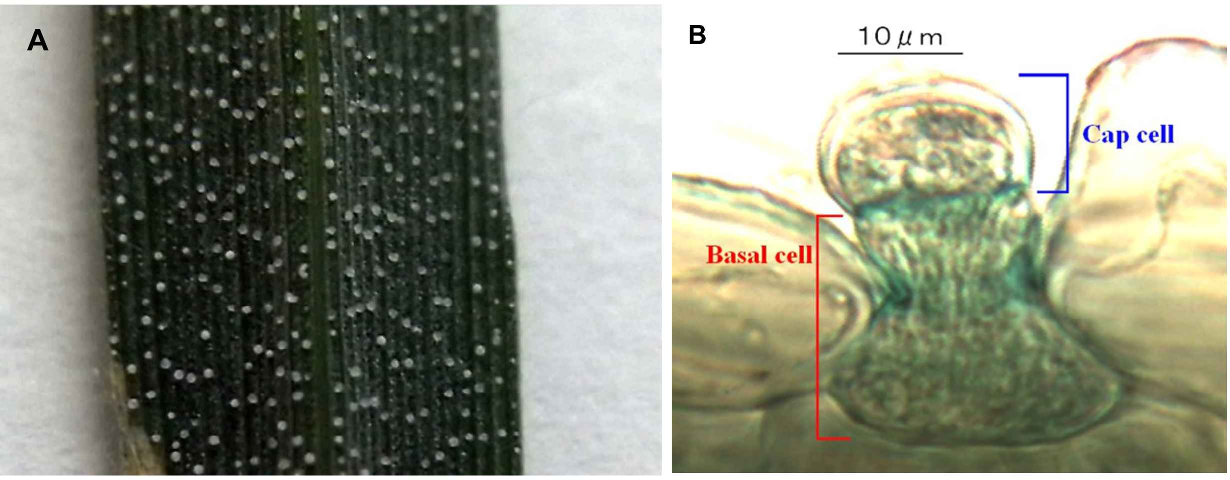


Fig. 1 Salts secreted on the leaf surface (A) and salt gland (B) of Rhodes grass (*Chloris gayana* Kunth).

Table 1 Poaceae plants whose ion secretion has been investigated.

Genus	Specific name	References
Subfamily Chloridoideae		
Aeluropus	*lagopoides*, *littoralis*, *pungens*	14, 18, 22
Bouteloua	*curtipendula*	13
Buchloë	*dactyloides*	13
Chloris	*gayana*	11
Cynodon	*dactylon*, *dactylon*×*transvaalensis*	21
Dactyloctenium	*aegyptium*	1
Dinebra	*retroflexa*	1
Distichlis	*spicata*	7
Eleusine	*indica*, *tristachya*	6
Enneapogon	*nigricans*	6
Eragrostis	*cilianensis*, *elongata*	6
Leptochloa (*Diplachne*)	*digitata*, *fusca*	4, 6
Odyssea	*paucinervis*	17
Pappophorum	*pappiferum*, *philippianum*	5
Spartina	*alterniflora*, *anglica*, *townsendii*	2, 9, 19
Sporobolus	*africanus*, *airoides*, *arenarius*, *cryptandrus*, *elongatus*, *spicatus*, *virginicus*	3, 6, 13, 16
Zoysia	*japonica*, *japonica*×*tenuifolia*, *macrantha*, *macrostachya*, *matrella*, *sinica*, *tenuifolia*	6, 12
Subfamily Arundinoideae		
Phragmites	*australis*	15
Rytidosperma	*linkii*	6
Subfamily Ehrhartoideae		
Oryza	*sativa*	10
Porteresia	*coarctata*	8
Subfamily Panicoideae		
Digitaria	*sanguinalis*	3
Imperata	*cylindrica*	6
Panicum	*repens*, *virgatum*	3, 6
Paspalum	*distichum*	3
Pennisetum	*clandestinum*	6
Spinifex	*hirsutus*	6
Zea	*mays*	20

1, Liphschitz and Waisel 1974; **2**, Rozema *et al.* 1981a; **3**, Liphschitz and Waisel 1982; **4**, Bhatti and Wieneke 1984; **5**, Taleisnik and Anton 1988; **6**, Amarasinghe and Watson 1989; **7**, Warren and Brokelman 1989; **8**, Flowers *et al.* 1990; **9**, Bradley and Morris 1991; **10**, Balakrishna 1995; **11**, Taleisnik *et al.* 1997; **12**, Marcum *et al.* 1998; **13**, Marcum 1999; **14**, Salama *et al.* 1999; **15**, Burke *et al.* 2000; **16**, Ramadan 2001; **17**, Somaru *et al.* 2002; **18**, Chen *et al.* 2003; **19**, Koyro and Huchzermeyer 2004; **20**, Ramadan and Flowers 2004; **21**, Marcum and Pessarakli 2006; **22**, Barhoumi *et al.* 2007.

ance, and these have not yet been reviewed. Therefore, this paper describes the phenomenon "ion secretion via salt glands in Poaceae" from various view points with a focus on recent studies.

SALT GLANDS IN POACEAE

Salt glands are found in many plant species. Their structures differ among plant species and are mainly divided into three types: the bladder cells of the Chenopodiaceae, the multicellular glands of dicotyledons and the bicellular glands of the Poaceae (Liphschitz and Waisel 1982; Fahn 1988; Thomson *et al.* 1988; Wahid 2003). In addition, unicellular epidermal structures of some Poaceae species reportedly have salt secretion ability (Bal and Dutt 1986; Flowers *et al.* 1990; Balakrishna 1995; Latha *et al.* 2004).

Ion secretion via active salt glands is considered an energy-consumptive process, because ions are transported against a concentration gradient; ion concentration in the fluid secreted by salt glands is greater than that in the plant tissue or the medium (Pollak and Waisel 1970; Ramati *et al.* 1976; Thomson *et al.* 1988). For efficient ion transport, active salt glands have some characteristic structures. This section outlines the structures and ion transport processes in the two kinds of salt gland in Poaceae, bicellular glands and unicellular glands (salt hairs).

Bicellular glands

Bicellular glands of the Poaceae are mainly found on the

Table 2 Sodium secretion via bicellular glands of Poaceae.

Species	**Na secretion rate[A] (μmol g^{-1}DW day^{-1})**	**NaCl treatment (mM)**	**References**
Subfamily Chloridoideae			
Aeluropus littoralis	600	100	Liu *et al.* 2006
	1000	300	Pollak and Waisel 1970
Bouteloua curtipendula	3	100	Liphschitz and Waisel 1974
	4	200	Murcum 1999
Buchloë dactyloides	5	200	Murcum 1999
Chloris gayana	270	150	Liphschitz and Waisel 1974
	550[B]	160	Kobayashi *et al.* 2007
Cynodon dactylon	7	150	Liphschitz and Waisel 1974
cv. ‘Sultan’	9	113	Murcum and Pessarakli 2006
cv. ‘FloraTex’	130	113	Murcum and Pessarakli 2006
Dactyloctenium aegyptium	320	100	Liphschitz and Waisel 1974
Dinebra retroflexa	27	50	Liphschitz and Waisel 1974
Distichlis spicata	270	500	Murcum *et al.* 2007
Eleusine indica	12	100	Liphschitz and Waisel 1974
Leptochloa fusca	290	250	Gorham 1987
	350[B]	50	Bhatti and Sarwar 1993
Spartina anglica	80	200	Rozama *et al.* 1981a
Sporobolus arenarius	25	150	Liphschitz and Waisel 1974
	450	200	Ramati *et al.* 1976
S. airoides	70	200	Murcum 1999
S. cryptandrus	5	200	Murcum 1999
Zoysia japonica	20	400	Marcum *et al.* 1998
Z. matrella	50	400	Marcum *et al.* 1998
	100	200	Marcum and Murdoch 1990
Subfamily Panicoideae			
Digitaria sanguinalis	2	150	Liphschitz and Waisel 1982
Panicum repens	4	150	Liphschitz and Waisel 1982
Paspalum distichum	6	150	Liphschitz and Waisel 1982
Zea mays	10	120	Ramadan and Flowers 2004

[A] Values are calculated from previously published data to be expressed as μmol g^{-1}DW day^{-1}.

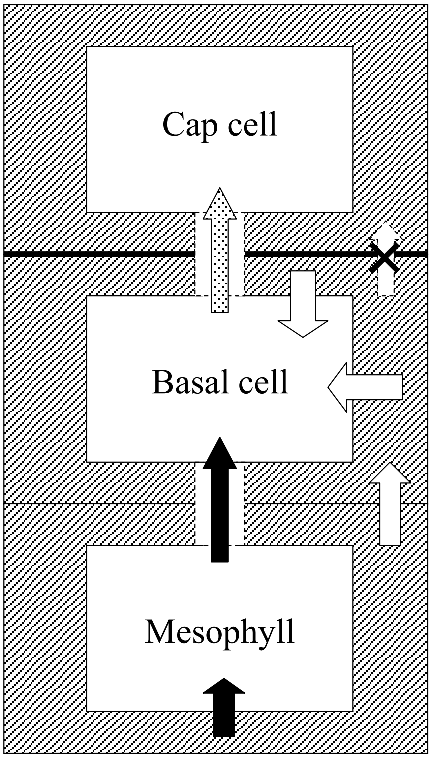
[B] Detached leaf experiment.

leaves (Amarasinghe and Watson 1989; Amarasinghe 1990), but also on other aerial parts such as flowering culms (Arriaga 1992). Bicellular glands are believed to exist in almost all Poaceae plants except for the subfamily Pooideae (Tateoka *et al.* 1959; Amarasinghe and Watson 1988, 1989; Ramadan and Flowers 2004), while their ability to secrete ions varies greatly (**Table 2**). However, the bicellular glands that possess a low ability to secrete ions also seem to act as glandular trichomes, because the secretion of metabolites such as polysaccharides and proteins has been detected with light microscopic histochemistry (Amarasinghe 1990; McWhorter *et al.* 1995).

The basic structures of bicellular glands are similar in Poaceae (Levering and Thomson 1971; Oross and Thomson 1982a; Oross *et al.* 1985; Amarasinghe and Watson 1988; Naidoo and Naidoo 1998b; Somaru *et al.* 2002). Bicellular glands look like trichomes or microhairs composed of two cells (**Fig. 1B**), which develop from a single protoderm cell (Skelding and Winterbotham 1939). One of the cells composing the bicellular gland is in contact with the leaf epidermis and mesophyll, and is called the “basal cell”, while another cell, which is in contact only with the basal cell, is called the “cap cell” (**Fig. 1B**). These two kinds of cells have different functions; the basal cell acts as the collecting cell, while the cap cell acts as the secreting cell (Liphschitz and Waisel 1982). The leaf cuticle extends over the outer surface of the glands, and the extracellular space formed between the cap cell and cuticle is called the collecting chamber, where ions emitted by the cap cell are accumulated (Levering and Thomson 1971; Oross and Thomson 1982a, 1984; Naidoo and Naidoo 1998b; Somaru *et al.* 2002). Continuing accumulation of ions increases the hydrostatic pressure of the solution in the collecting chamber, which stretches the cuticle and lets the solution escape through the pores formed by rupturing the pre-existing weak spots in the cuticle (Oross *et al.* 1985; Naidoo and Naidoo 1998b; Somaru *et al.* 2002).

The cells of active bicellular glands are distinguishable from other cells. They contains dense cytoplasm, many mitochondria, a large nucleus, some rough endoplasmic reticula, dictyosomes, free ribosomes, plastids, small vesicles and multivesicular structures (Liphschitz and Waisel 1982; Fahn 1988; Thomson *et al.* 1988). One of the characteristic ultrastructures of active bicellular glands is the numerous long invaginations of the plasma membranes in the basal cell, termed the partition membrane (Levering and Thomson 1971; Oross and Thomson 1982a, 1982b, 1984; Oross *et al.* 1985; Naidoo and Naidoo 1998b; Somaru *et al.* 2002). The partition membrane originates from the wall between the basal and cap cells, and penetrates deeply into the basal cell cytoplasm. The partition membrane amplifies the surface area, and forms the extracellular space which probably functions as an ion transport pathway. In addition, many mitochondria align between the partition membranes to supply energy for ion transport across the partition membrane. From these observations, the partition membrane is believed to play an important role in efficient ion secretion. This idea is supported by the fact that all plants that efficiently secrete salts via bicellular glands possess partition membranes in their basal cells (Amarasinghe and Watson 1989).

The ion transport pathway (**Fig. 2**), from the veins via bicellular glands to the exterior, is not fully clarified. There are some hypotheses about ion transport to the basal cell. One hypothesis is that ions are transported through apoplastic pathways and the partition membranes function in the absorption of ions from the apoplast (Oross *et al.* 1985). This hypothesis is supported by tracer studies indicating that an apoplastic continuum exists between the cell wall of mesophyll and the extracellular space formed by partition membranes in the basal cell (Oross and Thomson 1982b; Naidoo and Naidoo 2006). Another hypothesis is that ions are transported through symplastic pathways. This idea is supported by the presence of abundant plasmodesmata between the basal cell and surrounding mesophyll cells, the localization of Cl ions in the vicinity of the plasmodesmata



⇨**Apoplastic ion transport pathway to the basal cell.**

➡**Symplastic ion transport pathway to the basal cell.**

⇨**Symplastic ion transport from the basal cell to the cap cell.**

Fig. 2 A model diagram of ion transport pathway in ion secretion via bicellular glands of Poaceae. The apoplast is shown in hatched and the symplast in white. (1) Ions are transported to the basal cell through an apoplastic pathway (white arrows) and/or through a symplastic pathway (black arrows). (2) Ions are transported symplastically from the basal cell to the cap cell (dotted arrows), because there is apoplastic discontinuity between these cells (×).

and the detection of symplastic tracer in the gland cells (Naidoo and Naidoo 2006). In addition to these two hypotheses, the combination of apoplastic and symplastic pathways has been suggested (Naidoo and Naidoo 1999, 2006). This idea is partly supported by the fact that intense ATPase activity is detected at the plasma membrane and partition membranes in the basal cell, and at the plasmodesmata between the basal and mesophyll cells (Naidoo and Naidoo 1999). In contrast to the ion transport pathway to the basal cell, there is a common view of ion transport between the basal and cap cells; ions are transported symplastically through the plasmodesmata (Oross *et al.* 1985; Naidoo and Naidoo 1999, 2006). This view is supported by tracer studies revealing apoplastic discontinuity between the basal and cap cells (Oross and Thomson 1982b; Naidoo and Naidoo 2006).

Unicellular glands (salt hairs)

In some Ehrhartoideae species such as *Porteresia coarctata* (formerly *Oryza coarctata*) and *Oryza sativa*, a unicellular epidermal structure called the salt hair reportedly has salt secretion ability, and this unicellular structure is therefore regarded as a salt gland (Bal and Dutt 1986; Flowers *et al.* 1990; Balakrishna 1995; Latha *et al.* 2004). Unicellular salt glands are found on the adaxial surface of the leaf, and each unicellular gland has an electron-dense vacuole and is covered with cuticle and salts (Flowers *et al.* 1990). Bal and Dutt (1986) hypothesized that unicellular glands could eliminate ions by bursting themselves and that new glands are formed continuously in place of the bursted glands. However, detailed mechanisms of ion secretion via the unicellular gland remain unclear.

Although the structures that actually secrete salts are different among the Poaceae species (bicellular and unicellular glands), these structures probably co-exist on the same leaf in some plants. *Porteresia coarctata* and *Oryza sativa*, which possess unicellular glands, also have bicellular glands on their shoots (Tateoka 1963; Amarasinghe and Watson 1988). However, these bicellular glands seem to have a low ability to secrete ions, because partition membranes are absent in the bicellular glands of *O. sativa* (Amarasinghe and Watson 1988). *Leptochloa fusca*, which possesses active bicellular glands, also has swollen epidermal hairs on its leaves, which might contribute to salt regulation in the leaves (Bhatti *et al.* 1992).

IONS SECRETED VIA SALT GLANDS

Salt glands of Poaceae secrete a wide variety of ions. On the other hand, salt glands have a preference of ions for secretion, and similar trends of this preference are found within the subfamily (Pollak and Waisel 1970; Rozema *et al.* 1981b; Joshi *et al.* 1983; Naidoo and Naidoo 1998a). This preference probably enables the plants to secrete harmful ions efficiently and to retain the nutritional ions in their tissue. In this section, the secretion of individual ions and their interactions in the bicellular glands are described first, and then the ion secretion in the unicellular glands (salt hairs) is described.

Bicellular glands

Na, K, Ca, Mg

Sodium is the most preferred cation for secretion via bicellular glands (Liphschitz and Waisel 1982; Thomson *et al.* 1988), while Na secretion rate greatly differs among plant species (**Table 2**). High ability of bicellular glands to secrete Na is observed in the plants which belong to the subfamily Chloridoideae (**Table 2**), and it enables efficient Na elimination from the plant tissues. For example, the amount of secreted Na reaches to five times as much as that of retained Na in the leaves of *Distichlis spicata* under 100 mM NaCl treatment for 5 days (Liphschitz and Waisel 1982). Furthermore, the concentration of Na in the leaves of *Sporobolus spicatus* is greatly increased during the day (from 220-280 μmol g^{-1} FW at 0600 h to 390-580 μmol g^{-1} FW at 1800h), but the bulk of accumulated Na is secreted at night and the Na concentration in the leaves is similar at 0600 h of consecutive mornings (Ramadan 2001).

Potassium is reportedly found in the secreted salts, but its secretion efficiency is less than Na (Liphschitz and Waisel 1974; Bradley and Morris 1991; Marcum and Murdoch 1992; Warwick and Halloran 1992; Salama *et al.* 1999; Somaru *et al.* 2002). Treatment with KCl increases the secretion of K, but an increase in K secretion is 15-60% of that in the Na secretion elicited by isoosmolar treatment of NaCl (Pol-lak and Waisel 1970; Rozema *et al.* 1981b; Wieneke *et al.* 1987; Kobayashi *et al.* 2007).

Calcium is also found in the secreted salts, but the amount of secreted Ca is small (Wieneke *et al.* 1987; Naidoo and Naidoo 1998a; Salama *et al.* 1999; Somaru *et al.* 2002; Koyro and Huchzermeyer 2004; Kobayashi *et al.* 2007). Treatment with $CaCl_2$ increases the Ca secretion, but the increase in the Ca secretion is 5-15% of that in the Na secretion elicited by isoosmolar treatment of NaCl (Pollak and Waisel 1970; Rozema *et al.* 1981b; Wieneke *et al.* 1987).

Although magnesium is the second major cation in sea water, the amount of secreted Mg is quite small (Naidoo and Naidoo 1998a; Salama *et al.* 1999; Somaru *et al.* 2002; Koyro and Huchzermeyer 2004; Kobayashi and Masaoka 2008). Treatment with $MgCl_2$ to *Chloris gayana* increases the Mg secretion in both whole plant experiments and detached leaf experiments, but the increase in Mg secretion is about 1% of that in the Na secretion elicited by isoosmolar treatment of NaCl (Kobayashi *et al.* 2007; Kobayashi and Masaoka 2008).

In general, bicellular glands in the Chloridoideae have a selective secretion in the order of Na >> K > Ca > Mg (Joshi *et al.* 1983; Naidoo and Naidoo 1998a; Ramadan 2001), and this preference is different from those in some dicotyledonous plants such as *Armeria maritima* (K > Ca > Na, Rozema *et al.* 1981b). Because of the preference of ions for secretion, the ratio of ions in the secreted salt is greatly different from that in the plant tissue; the proportion of Na in the secreted salt is far greater than that in the plant tissue (Ramati *et al.* 1976; Ramadan 2001; Ramadan and Flowers 2004). However, the mechanisms causing these preferences are not yet fully clarified. Kobayashi *et al.* (2007) indicate that the secretion mechanisms of Na and K differ in *Chloris gayana*, at least partially, because some ion transport inhibitors have different effects on Na and K secretion. Therefore, some specific mechanisms seem to contribute to high ability of Na secretion in Chloridoideae plants.

Anions (Cl, SO_4, NO_3, PO_4)

Chloride is the most preferred anion for secretion, and its secretion rate is comparable to that of Na (Ramati *et al.* 1976; Wieneke *et al.* 1987; Taleisnik and Anton 1988; Marcum and Murdoch 1994; Sargeant *et al.* 2006; Barhoumi *et al.* 2007; Kobayashi *et al.* 2007). In *Leptochloa fusca*, the high ability of bicellular glands to secrete Cl continues throughout most of the life span of a leaf and prevents a substantial increase in Cl concentration of the leaf as leaf age increases (Klagges *et al.* 1993).

SO_4 is found in the secreted salts, but the amount of secreted SO_4 is small (Bradley and Morris 1991; Klagges *et al.* 1993; Wu *et al.* 1997; Ramadan 2001). Treatment with 50 mM Na_2SO_4 to *Distichlis spicata* increases the secretion of SO_4, but this increase is about 6% of that in Cl secretion elicited by 50 mM NaCl (Wu *et al.* 1997).

NO_3 is detected in the salts secreted on the leaf of *Leptochloa fusca* and *Chloris gayana* (Klagges *et al.* 1993; Kobayashi and Masaoka 2008), but Salama *et al.* (1999) reported that they could not detect NO_3 in *Aerulopus lagopoides*. Treatment with 40 mM $Mg(NO_3)_2$ to the detached leaves of *C. gayana* increases the secretion of NO_3, but the increase in NO_3 secretion is about 50% of that in Cl secretion elicited by 40 mM $MgCl_2$ (Kobayashi and Masaoka 2008).

A small amount of PO_4 is detected in the salts secreted on the leaf of *Spartina alterniflora* and *Aerulopus pungens* (McGovern *et al.* 1979; Chen *et al.* 2003), but is not detected in *Leptochloa fusca* and *Sporobolus spicatus* (Klagges *et al.* 1993; Ramadan 2001). Since there is no report examining PO_4 secretion under PO_4 treatment, it remains unclear whether the small amount of secreted PO_4 is caused by a low ability of bicellular glands to secrete PO_4 or by a low concentration of PO_4 in the plant tissue. In *S. alterniflora* that grow in tidal marshes, it is reported that the proportion of PO_4 in the secreted salts is much higher than that in seawater while the proportion of SO_4 in the secreted salts is much less than that in seawater (McGovern *et al.* 1979). Therefore, PO_4 might be preferable for secretion than SO_4 in some plant species.

Heavy metal and potentially toxic ions

Salts secreted via bicellular glands reportedly contain heavy metal and potentially toxic ions, such as Fe in *Aeluropus pungens* (Chen *et al.* 2003), Se in *Distichlis spicata* (Wu *et al.* 1997), Fe, Mn and Zn in *Spartina anglica* (Rozema *et al.* 1991) and Cd, Cr, Cu, Hg, Ni, Pb and Zn in *Spartina alterniflora* (Kraus *et al.* 1986; Kraus 1988; Burke *et al.* 2000; Windham *et al.* 2001).

In *Distichlis spicata*, selenium secretion via bicellular glands is increased by 2 ppm (25 μM) of Se treatment, and there are some ecotypical differences in Se secretion (Wu *et al.* 1997). Ion secretion efficiency for Se is almost similar to that for SO_4 in *D. spicata*, and Se uptake and secretion is inhibited by Na_2SO_4 treatment (Wu *et al.* 1997).

In *Spartina alterniflora*, active bicellular glands enable it to secrete Cu, Hg and Zn more efficiently than co-existing *Phragamites australis* (Burke *et al.* 2000; Windham *et al.* 2001). In addition, *S. alterniflora* prefers some metal ions for secretion; it secretes Cd and Ni more efficiently than Pb, Cr and Cu (Kraus 1988).

Interactions between ions

In addition to the concentration and secretion efficiency of individual ions, co-existing ions can affect ion secretion. Concerning cation-anion interactions, ion secretion is affected by the secretion efficiency of the counter ion. Na secretion is greater in the treatment of NaCl than Na_2SO_4 (Wu *et al.* 1997), and Cl secretion is greater in the treatment of NaCl than KCl or $CaCl_2$ (Rozema *et al.* 1981b; Wieneke *et al.* 1987; Kobayashi *et al.* 2007).

Between cations, both inhibiting and stimulating effects have been reported. In *Aerulopus littoralis*, *Spartina anglica* and *Leptochloa fusca*, antagonistic relationships are observed among Na, K and Ca (Pollak and Waisel 1970; Rozema *et al.* 1981b; Wieneke *et al.* 1987). On the other hand, there are some reports of stimulating effects. Treatment with NaCl increases the secretion of K, Ca and Mg in *Spartina townsendii*, but the factors causing these increases are not documented (Koyro and Huchzermeyer 2004). Treatment with $MgCl_2$ to *Chloris gayana* significantly increases both Mg and K secretion, but the increase in K secretion is more than 50 times higher than for Mg secretion (Kobayashi and Masaoka 2008). The authors suggest that K is secreted in place of Mg under $MgCl_2$ treatment, because K is secreted more preferentially than Mg and is abundant in the leaves.

Unicellular glands

The salts secreted via unicellular glands reportedly contain Na, K, Ca, Mg and Cl (Bal and Dutt 1986; Flowers *et al.* 1990; Balakrishna 1995). Sodium is the most preferred cation for secretion (Bal and Dutt 1986), and the amount of secreted Na reaches to as much as that of accumulated Na in *Porteresia coarctata* under 25% seawater treatment for 6 or 29 days (Flowers *et al.* 1990). The amount of secreted Cl is comparable to that of Na in *P. coarctata* (Bal and Dutt 1986). The secretion of K and Ca is always less than half of the Na secretion, but the secretion of Mg is greatly increased by the treatment with saline river water and reaches to 70% of Na secretion in *P. coarctata* (Bal and Dutt 1986).

FACTORS AFFECTING ION SECRETION

Ion secretion via salt glands is affected by various environmental conditions and treatments. In this section, the factors affecting ion secretion in bicellular glands are described, since these factors have not been examined in unicellular glands yet.

Ion secretion via bicellular glands is affected by environmental conditions, such as temperature, humidity, light and redox potential of the soil. Salt secretion increases with increasing air temperature in *Leptochloa fusca*; salt secretion at 39°C is about 20-times and 3-times more than that at 19°C and 32°C, respectively (Gorham 1987). The efficiency of Na secretion (secreted Na / increase in Na concentration in the leaves) is higher with high air humidity than with low air humidity in *Aeluropus littoralis* (Pollak and Waisel 1979). Light increases the amount of secreted salt in *A. littoralis* and *L. fusca* (Pollak and Waisel 1979; Bhatti and Sarwar 1993). This light-induced increase in salt secretion may be caused by an increase in the energy supply for salt secretion (Bhatti and Sarwar 1993), or by an increase in the salt supply with increasing transpiration stream (Pollak and Waisel 1979). The secretion of Fe and Mn via bicellular glands of *Spartina anglica* is related to the redox potential of soils, which affects the solubility of these metals in the soil (Rozema *et al.* 1991). Under field conditions, salt secretion shows diurnal patterns in *Sporobolus spicatus*; Na secretion rates are lowest between 0900 and 1500 h and tend to increase gradually until the following morning (Ramadan 2001). The diurnal patterns in which salt secretion is high at night and low during the day were also reported in *Distichlis spicata* (Hansen *et al.* 1976). Although the environmental factors causing these diurnal patterns are not fully clarified, Pollak and Waisel (1979) suggest that the prevailing high air humidity and the decrease of water stress will be advantageous for night secretion.

Salt in the medium itself may affect ion secretion. The treatments with NaCl (40-400 mM) or seawater (80%) increase the density of bicellular glands (gland number / leaf area) in *Odyssea paucinervis*, *Zea mays* and *Aeluropus littoralis* (Somaru *et al.* 2002; Ramadan and Flowers 2004; Barhoumi *et al.* 2007). However, the density of bicellular glands is not increased by NaCl treatments (200-400 mM) in *Chloris gayana* and *Zoysia* species (Liphschitz *et al.* 1974; Marcum *et al.* 1998). Ramadan and Flowers (2004) indicate that the increase in the density of bicellular glands under salt treatment is partly due to its reducing effect on leaf area in *Zea mays*, but the factors causing these different responses to the salt treatment among the species remain unclear.

Ion secretion can be controlled by chemicals such as plant hormones and ion transport inhibitors. Application of abscisic acid (ABA) to the detached leaves of *Leptochloa fusca* significantly reduces Na secretion (Wieneke *et al.* 1987). Because application of ABA has no significant effect on Na concentration in the leaves, ABA appears to affect the Na-secretion process rather than the Na-uptake process. Application of benzyl adenine, a member of cytokinins, increases the number of bicellular glands in *Chloris gayana* and *Zea mays* (Liphschitz *et al.* 1974; Ramadan and Flowers 2004). Bhatti and Sarwar (1993) reported that application of dinitrophenol (an inhibitor of H^+-ATPase) to the detached leaves of *Leptochloa fusca* increases ion secretion at a low concentration (0.1-0.5 mM), but inhibits at a high concentration (above 1.0 mM). Application of five ion transport inhibitors [barium ion (an inhibitor of various cation channels and transporters), orthovanadate (an inhibitor of plasma membrane H^+-ATPase), ouabain (an inhibitor of Na^+-K^+-ATPase in animal cells), tetraethylammonium (TEA, an inhibitor of the K^+ channel) and verapamil (an inhibitor of the K^+ channel and Ca^{2+} channel)] to the detached leaves of *Chloris gayana* significantly reduces Na secretion under NaCl treatment (Kobayashi *et al.* 2007). Application of ouabain, TEA and verapamil to the detached leaves of *C. gayana* significantly reduces K secretion under KCl treatment, but application of barium ion and orthovanadate have no significant effect (Kobayashi *et al.* 2007). Furthermore, application of ouabain, TEA and verapamil to the detached leaves of *C. gayana* also inhibits the increase in K secretion by $MgCl_2$ treatment (Kobayashi and Masaoka 2008).

EFFECTS OF ION SECRETION ON CROP QUALITY AND ENVIRONMENT

As mentioned above, active ion secretion via salt glands has a role in the salinity and heavy metal tolerance of plants, and therefore contributes to the plant growth on saline or heavy metal-contaminated soils. Because ion secretion is the process to release element that plants contain in their tissues to the outside, it also has some impacts on the nutritive value of plants and the surrounding environment.

An increase in the salt concentration of fields is one of the most serious agricultural problems worldwide. In salt-affected fields, the cultivation of salinity-tolerant crops is recommended for crop production and soil reclamation (Kumar 1996; Qadir *et al.* 1996). However, an increase in salt concentration of the crops can decrease the crop quality. For example, a high concentration of Na in the diet depresses livestock production (Masters *et al.* 2005a, 2005b). Since salt-secreting Poaceae can maintain Na concentration to a low extent, they are considered as a valuable fodder and hay for livestock even in salt-affected fields (Kumar 1996).

The effects of ion secretion on the surrounding environment have mainly been examined in *Spartina alterniflora*, and both positive and negative effects have been reported. *S. alterniflora* appears to act as a source of P to the salt marsh ecosystem; it absorbs P from the soil, transports it to the shoots and releases it to the estuarine and coastal water via salt glands (Reimold 1972; McGovern *et al.* 1979). In the heavy metal-contaminated sites, *S. alterniflora* also releases heavy metals through a similar process (Kraus *et al.* 1986; Kraus 1988; Burke *et al.* 2000; Windham *et al.* 2001). Because the release of heavy metal via salt glands can increase bioavailabilitiy of heavy metals within estuaries, it is suggested that the restoration efforts that replace *Phragmites australis*, which have a low ability to secrete ions, with *S. aletrniflora* may not be desirable at sites where sediment metal contamination is substantial (Burke *et al.* 2000; Windham *et al.* 2001).

CONCLUSION AND FUTURE RESEARCH

Salt glands are believed to exist in almost all Poaceae species except for the subfamily Pooideae, while there is diversity in their ion-secretion ability. Because active ion secretion via salt glands contributes to the reduction of toxic ion concentration in plant tissue, the salinity and heavy metal tolerance of Poaceae plants possessing inactive salt glands can be improved by the introduction of active ion secretion mechanisms to their salt glands. Therefore, it is important to clarify the mechanisms of ion secretion in active salt glands. However, these mechanisms have not yet been fully clarified. Especially, there are few studies on the molecular basis of ion secretion via salt glands. Therefore, further research on ion secretion mechanisms, including their molecular basis, will be needed. Information obtained by recent studies, such as the difference in salt secretion ability among the cultivars and the preference of ions for secretion, will be valuable clues to clarify the ion secretion mechanisms in the salt glands of the Poaceae.

Ion secretion via salt glands has diverse aspects other than a tolerance mechanism. Because ion secretion via salt glands has some impacts on crop quality and element circulation as mentioned in this review, this phenomenon will be applicable for agricultural and environmental purposes. Therefore, the development of practical techniques using ion secretion via salt glands for these purposes is also expected.

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JAPANESE ABSTRACT

土壌の塩類集積は植物の生育を阻害する要因であり、塩類集積に対応するために植物は様々な機構を発達させている。塩類腺からの塩類排出もその一つであり、塩類腺は地上部中の過剰塩類を体外に排出する役割を果たしているとされている。塩類腺からはナトリウムや塩素以外のイオンも排出され、重金属集積土壌で生育した植物からは重金属が排出されることが報告されている。塩類腺からのイオン排出は、耐性機構としての役割に加えて、土壌から吸収した無機成分を周辺環境に放出することによる物質循環や、ミネラル含有率など作物の栄養価にも影響を与えている。イネ科植物においては、塩類排出能力には大きな相違があるものの、イチゴツナギ亜科を除くほぼ全ての種に塩類腺が存在するとされている。本総説では、イネ科植物の塩類腺について、その構造や排出メカニズムなどの概要を解説する。