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General

Sporobolus stapfianus plants form perennial tufts; narrow leaf blades, about 15 cm long, arise close to the soil surface. The diffuse inflorescences may reach 40cm in height. Clayton (1974) presents a full description of the species. *S. stapfianus* leaves have the Kranz leaf anatomy typical of phosphoenolpyruvate carboxylase (PCK) type C_4-pathway photosynthesis, as do all desiccation tolerant grass species (Sutaryono 1992). C_4-photosynthesis is associated with (a) adaptation of plants to a warm climate (the distribution of *S. stapfianus* is mainly tropical) and (b) water use efficiency that would assist an ancestral species to exploit arid sites. Plants flower and set seed in the middle of the wet summer season (January, February) in South Africa. Caryopses are small, ~0.5 mm long, – a size that would allow their transport in water, in wind and on animals.

S. stapfianus grows on sandy or shallow soils in grassland or open deciduous bushland; often where water collects in rock pans or on compacted soils; 600–2300 m altitude, from equatorial Africa (Nigeria, Uganda and Ethiopa) south to Botswana and the subtropical regions of South Africa (Clayton 1974). The growth period of *S. stapfianus* is mainly in the warm to hot wet season. During the long warm dry 'winter' period *S. stapfianus* plants dehydrate and survive in an anabiotic state. Much of the distribution is at altitudes over 1500 m where nights are frosty in 'winter'. *S. stapfianus* plants cultivated in the open in Australia grew successfully in tropical areas (~12°S to ~24°S), more so than other resurrection grasses tested; however, *S. stapfianus* did not survive the cool wet winters in temperate areas at ~35°S to ~38°S (Gaff *et al.* unpubl. data).

Several other *Sporobolus* spp. also possess desiccation tolerant foliage: *S. festivus, S. pellucidus* Hochst and *S. lampranthus* Pilger in Africa; *S. blakei* De Nardi and *S. elongatus* R.Br. in Australia and *S. atrovirens* (Kunth)Kunth in Mexico (Gaff 1971, 1977; Gaff and Ellis 1974; Gaff and Latz 1978; Iturriaga *et al.* 2000). The genus *Sporobolus* is in the Tribe Cynodonteae, Subfamily Chloridoideae, Family Poaceae (GPWG 2001; Kellog 2002). The Tribe Cynodonteae includes *inter alia* six genera, which contain some desiccation-tolerant species. Since the large genus *Sporobolus* has only seven species with desiccation tolerant leaf tissue (Gaff and Ellis 1974; Gaff and Latz 1978), desiccation-tolerant foliage may have evolved recently in *Sporobolus* and probably independently of its evolution in other genera. Desiccation tolerance is restricted to the basal centimetre of the leaves of three *Sporobolus* species – possibly an indication of an early stage of evolution of the 'resurrection' strategy. The young fully-expanded leaves of *S. stapfianus*, however, express desiccation tolerance along the full leaf lamina, but senescent leaf tissue is sensitive to desiccation (Gaff and Ellis 1974).

Synonymy: *S. stapfianus* Gandoger was split from *Sporobolus festivus* A.Rich. Our paper employs the usage of *S. festivus sensu stricto* for the taxon remaining after removal of *S. stapfianus*. The names *Sporobolus festivus* A.Rich. var. *stuppeus* Stapf and *S. stuppeus* (Stapf) Stent. are no longer met as synonyms for *S. stapfianus*. *S. stapfianus* has woolly brown hairs at the base of the leaf sheath; the hairs are not present on *S. festivus sensu stricto* (Chippindall 1955; Clayton 1974).

Cell fine structure

As for the dicot resurrection bush *Myrothamnus flabellifolia,* the fine-structural response of desiccation tolerant *S. stapfianus* leaf cells to drying is one of maintenance of most of their lipoprotein membrane structure as plants dried; thylakoid and grana were numerous (Altus and Hallam 1980; Quartacci *et al.* 1997; Dalla Vecchia *et al.* 1998). Mitochondria were also clearly seen. The live dry cells displayed a fragmentation of the vacuole into numerous small vacuolar areas and a marked decline in starch grain size and number (Quartacci *et al.* 1997). Live leaves re-established normal cell fine structure and starch complements within 2 days of rehydration. In leaves dried detached, 'altered chloroplasts … retained large quantities of starch and lipid-like inclusions in the stroma…(and)…Vacuolar areas remained larger and undefined. ER-like vesicles were not recognizable' (Quartacci *et al.* 1997). In this comparison, both live and dead samples were dry, therefore perceived differences in electronmicrographs of live and dead tissue are less likely to be dry-state artefacts than in comparisons of dry leaves against hydrated leaves. (see also Lipids below). The walls of desiccation-tolerant *S. stapfianus* mesophyll cells folded as leaves dried; wall folding together with the mesh of numerous small vacuoles may reduce the risk of mechanical damage to the drying protoplast (Farrant 2007). Wall folding in the desiccation-tolerant dicot *Craterostigma wilmsii* was associated with less glucose and more galactose in wall xyloglucans as leaves dried (Farrant 2007).

Live airdry leaves retain little starch (0–17% of the starch content in hydrated *S. stapfianus* leaves; Gaff 1989; Quartacci *et al.* 1997). However, high starch contents were visible in micrographs of leaves dried detached (dead) (Quartacci *et al*. 1997): those authors suggested that sugar contents were too low to stabilize membranes during drying and that membrane damage contributed to cell death.

Lipid composition

Loss of membrane components was slightly less in drought-killed *S. stapfianus* leaves than in live airdry leaves (Quartacci *et al*. 1997): 33% loss of chlorophyll content (cf. ~55% in live leaves); 20% of lipid content (cf. ~27% in live leaves). Contents of monogalactosyldiacyl- glycerol (MGDG), a thylakoid electron transporter, fell ~72% in both live and dead leaves (Table 1). 'The reduced MGDG amount, its lower unsaturation and the low level of linolenic acid on desiccation …may have contributed to the maintenance of membrane fluidity in both dehydrated leaves to a level consistent with metabolic recovery on rewatering' (Quartacci *et al.* 1997).

Contrary changes in contents in *S. stapfianus* leaves drying alive compared with leaves injured during drying (Quartacci *et al.*1997) were found for: carotenoids (quenchers of damaging free-radicals) rose ~5% in live leaves, *cf.* ~15% decrease in dying leaves; phospholipids and conjugated dienes increased in live drying leaves (~25% increase; *cf.* ~33% decrease); phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were greater in live leaves but less in dead leaves. The augmented phospholipid may assist re-establishment of membrane integrity on rehydration of the live dry leaves.

Table 1. Lipid composition (mole %) of *Sporobolus stapfianus* leaves before and after dehydration (a) drying attached to intact plants (in bold print) or (b) drying detached, and after 24 h rehydration in both cases

Based on Quartacci *et al.* (1997). DG, diacylglycerols; DGDG, digalactosyldiacylglycerol; FFA, free fatty acids; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SQDG, sulphoquinovosyldiacyl-glycerol; TG, triacylglycerols; TPL, total polar lipids

	(a) Hydrated attached leaves	(b) Detached leaves kept hydrated	**(a)** Leaves dried attached	(b) Leaves dried detached	**(a)** Rehydrated (after leaves dried attached)	(b) Rehydrated (after leaves dried detached)
MGDG	**24**	30	**5.5**	7.7	**15**	3.1
DGDG	**17**	19	**15**	19	**14**	5.4
SQDG	**12**	7.3	**8.6**	7.7	**11**	6.5
PG	**8.8**	7.2	**6.8**	7.8	**8.0**	4.4
PC	**4.7**	5.7	**7.2**	4.6	**7.0**	2.6
PE	**4.7**	7.8	**7.9**	2.8	**6.3**	ND
FFA	**7.2**	9.0	**11**	9.3	**8.7**	26
TG	**17**	7.6	**29**	33	**23**	35
DG	**5.3**	6.3	**9.2**	8.3	**7.5**	17
MGDG / DGDG	**1.4**	1.5	**0.4**	0.4	**1.1**	0.6
TPL / FFA	**9.8**	8.6	**4.6**	5.4	**6.9**	0.8

Lipids during rehydration

On rehydration, the attached (live) leaves recovered the lipid composition of normal hydrated leaves (Quartacci *et al.* 1997). In desiccation-sensitive leaves, however, the lipid degradation during drying continued during rehydration; contents of free fatty acid, diacylglycerols and triacylglycerols increased markedly and some peroxidation of polar lipids occurred. Quartacci *et al.* (1997) concluded that failure of lipid synthesis to recover on rehydration prevented repair of essential membranes and that a lack of lipid synthesis on rehydration jeopardized survival more than did the changes in lipid composition during drying.

Overview of desiccation tolerance in *Sporobolus stapfianus*

Desiccation tolerance is acquired by leaves of the tropical resurrection grass *Sporobolus stapfianus* as they experience drought stress while they are attached to an intact plant, but not in leaves that have been detached before they have dried to 60% RWC. This contrast in the behaviour in leaves of the one species provides a valuable system for investigating the mechanism of desiccation tolerance in tissues with identical DNA.

In detached-dried leaves, lipid complements undergo partial recovery during rehydration, but this may be insufficient for full recovery of the leaf. Moreover, soluble protein contents fall markedly in leaves drying detached. There is evidence of oxidative stress during the dehydration of the desiccation-sensitive *S. stapfianus* leaves: contents of hydrogen peroxide and of dehydroascorbate both increase. However, ELIP protein may protect plastids against injury from ultraviolet radiation.

Also carotenoid contents are maintained and the antioxidant glutathione-ascorbate cycle enzymes display 30 to >100% of the activity in hydrated control samples, i.e. mechanisms for quenching and detoxifying free-radicals survive dehydration, but this does not ensure cell-revival on rehydration.

Desiccation tolerance in *S. stapfianus* is associated with accumulation of substances that are thought to protect cell membranes and proteins. Protective substances in *S. stapfianus* include particularly sucrose (possibly assisted by interaction with raffinose and trehalose) and LEA-proteins (mainly following changes in the conformation of the LEA at the high solute concentrations in airdry tissue).

Desiccation tolerance in *S. stapfianus* is also associated with major changes in the complement of proteins, both loss and gain of specific proteins. Novel proteins are synthesized in two phases during drying: the first (85–50% RWC) includes the onset of desiccation tolerance in attached drying leaves; the second phase (37–3% RWC) may provide particular proteins that support the initial stage of recovery during rehydration. For these changes to occur, hydolysis of protein and protein synthesis must continue at low RWCs.

The role of phytohormones in the induction of desiccation tolerance in leaves drying on *S. stapfianus* intact plants is not clear. Exogenous MJA and BR are the most effective phytohormones in stimulating the protoplasmic drought tolerance of free mesophyll cells of this species; their effects on PDT are quite insufficient for desiccation tolerance. ABA is much less effective than MJA or BR. Exogenous MJA, BR or ABA stimulates changes in the protein complement. Many ABA-responsive genes are expressed during the induction of desiccation tolerance (in leaves drying on intact plants), often before a marked increase in endogenous ABA content in *S. stapfianus* leaves in the late phase of drying.

Desiccation-tolerant leaves retain high contents of ATP in the dry state. This energy reserve supports the resumption of cell metabolism during rehydration; additional ATP is soon supplied by the rapid recovery of respiration in desiccation-tolerant plants.

S. stapfianus foliage possesses some drought avoidance mechanisms. The rate of drying presumably is slowed by epicuticular wax and by the tight inrolling of drying leaves (sealing off the adaxial surface and most of the stomata). Death of drying senescent leaves further reduces the area of transpiring foliage. Photooxidative stress is reduced by self-shading.

References in addition to those in the main paper

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