ROLE OF EPICUTICULAR WAX IN ADAPTATION TO SALINITY STRESS IN PIGEON PEA AND GINGELLEY[1] /

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Resumen

Plantas de gandul y ajonjolí al crecer bajo condiciones de salinidad con NaCl mostraron una gran acumulación de grasa epicuticular, la cual aumentó con la maduración de las hojas. La acumulación de grasa epicuticular se asoció con una reducción de la transpiración cuticular bajo condiciones de salinidad. La reducción de la transpiración cuticular pudo determinarse por la presencia de altos contenidos de alcoholes primarios y secundarios y aldehidos. Se concluye que la acumulación de grasa epicuticular juega un papel relevante en atenuar el daño causado por la salinidad al reducir la transpiración cuticular como medio de adaptación.

Introduction

Stomatal closure reduces the loss of water from the leaves which is of special importance when water uptake from the soil is diminished or if the evaporative demand is high. If the availability of soil water is severely reduced, the only way for a higher plant to survive is to conserve sufficient water in the tissues. The main pathway for transpiration then is through the cuticle. The importance of the cuticle for survival during water stress has already been reported (10). Environmental factors like light intensity, temperature and humidity affect the cuticle development and wax deposition (1). Reports on changes in content and chemical composition of epicuticular wax and its role in plants growing under salinity are scanty. In the present study an attempt has been made to find out (a) whether moderate salinity causes an adaptive reduction in cuticular transpiration; and (b) the relationship between cuticular transpiration and amount and composition of epicuticular wax in pigeon pea and gingelley plants.

Materials and methods

After a preliminary screening of pigeon pea (*Cajanus indicus* Spreng var. LRG-30) and gingelley (*Sesamum indicum* L. var TMV-1), for levels of salinity (0.1% to 0.6% NaCl), a differential response to 0.4% salinity level was found, pigeon pea showing tolerance and gingelley being susceptible. The seeds, after surface sterilization with 0.1% $HgCl_2$ for 2-3 min, were sown in 18 cm earthenware pots containing soil and manure in the ratio of 3:1. Thinning was done to 3 plants per pot before giving the salt treatment. The salt treatments were given at two stages of growth, 15 and 30 days after sowing. The salt content of the soil was raised to 0.4% salinity level by adding NaCl solution to the soil on air dry weight basis. The pH of the soil was maintained at 7.2 ± 0.1. The plants were grown under natural photoperiod. The leaf material (first formed trifoliate leaf from pigeon pea and first pair of leaves from gingelley) was collected at the following stages of growth for wax analysis and cuticular transpiration determination:

Stage 1: 7 days after first treatment (when the leaves showed full opening);

Stage 2: 15 days after first treatment (active period of growth);

Stage 3: 7 days after second treatment (maturation phase); and

Stage 4: 15 days after second treatment (initiation of senescense).

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As the plants showed some recovery symptoms at stage 2 in their physioological changes induced by salinity, an additional salt treatment was therefore given to maintain the same level of inhibition and to elicit a clear response from the plants (5).

Extraction and quantitative estimation of epicuticular wax were carried out colorimetrically (4). For qualitative studies, the wax components were fractionated by TLC as previously described (9). The individual wax components were identified by running purified *Brassica* wax components simultaneously.

Cuticular transpiration (CT) was determined according to Bengtson *et al.* (2) with a slight modification. Triplicate samples were analysed for each experiment.

Results and discussion

Levels of epicuticular wax (EW) and its chemical composition are shown in Tables 1 and 2, for pigeon pea and gingelley respectively. Total wax content increased in both the plants under salinity, the greater accumulation being observed in pigeon pea. An increase of 22.5, 22.7, 43.8 and 73.8 per cent in pigeon pea and 19.6, 22.1, 27.1 and 31.6 per cent over the controls in gingelley was observed at stages 1, 2, 3 and 4 respectively under salinity.

Fatty acids, primary and secondary alcohols, OH–β–diketones, β–diketones, aldehydes, hidrocarbons, esters and ketones and two unidentified spots (Un-1 and Un-2) were observed in pigeon pea under salinity. OH–β–diketones and Un-2 were found to be absent in control pigeon pea plants. On the other hand, β–diketones, OH–β–diketones and Un-1 were found to be absent in gingelley both under control and saline conditions. The fatty acid content was found to be high (on spot density basis) in controls, while primary and secondary alcohols and aldehydes were found to be high in the leaves of salinized plants.

Cuticular transpiration (CT) of the controls as well as salinized plants decreased with age; higher rates were observed in the controls than in the salinized plants. CT showed a decrease of 40.2, 38.9, 40.1 and 42.8 per cent in pigeon pea, and 28.9, 27.2, 31.9 and 31.3 per cent over the controls in gingelley were observed at stages 1, 2, 3, and 4, respectively, under salinity conditions (Table 2).

Salinity is known to affect many aspects of metabolism and to induce morphological and physiological changes, considered to be adaptive and to increase the chances of the plants to endure the stress imposed by salinity. The stunted growth of plants under saline conditions may even be traced to "physiological drought", a shortage of water within the plants when grown in moist but saline media. The lowered osmotic potential of the soil water resulting from high concentration of soluble salts was thought to prevent uptake of water by the plants. Thus any changes leading to a reduction of water loss or increase in water supply represent advantageous factors during salinity stress. Accumulation of leaf epicuticular wax and reduction of CT may thus play an important role in minimising water loss under salinity. It was found that increased wax on soybean leaves was accompanied by reduced CT,

Table 1. **Effect of NaCl salinity on epicuticular wax content and chemical composition in pigeon pea leaves (mg/dm² leaf area).**

Stage	Wax content	Fatty acids	OH-β-di-ketones	Un-1	Primary alcohols	Un-2	Secondary alcohols	β-di-ketones	Aldehydes	Hydrocarbons Esters and Ketones
					Control					
1	8.80 ± 0.03	+	–	Tr	+	–	Tr	+	Tr	+
2	9.95 ± 0.06	+	–	Tr	+	–	Tr	+	Tr	+
3	10.21 ± 0.05	+	–	Tr	+	–	Tr	+	Tr	+
4	9.18 ± 0.04	+	–	Tr	+	–	Tr	+	Tr	+
					Salinized					
1	10.78 ± 0.07	+	+	+	+ + +	+ +	+ +	+	+	+
2	12.21 ± 0.04	+	+	+	+ + +	+ +	+ +	+	+	+
3	14.68 ± 0.04	+	+	+	+ + +	+ +	+ +	+	+	+
4	15.95 ± 0.08	+	+	+	+ + +	+ +	+ +	+	+	+

– = absent; Tr = trace; + = present; + + = moderate; + + + = high.

Table 2. Effect of NaCl salinity on epicuticular wax content and chemical composition in gingelley leaves (mg/dm² leaf area).

Stage	Wax content	Fatty acids	Primary alcohols	Un-2	Secondary alcohols	Aldehydes	Hydrocarbons Esters and Ketones
			Control				
1	4.24 ± 0.09	+ +	+	+	+	+	+
2	4.39 ± 0.04	+ +	+	+	+	+	+
3	4.68 ± 0.04	+ +	+	+	+	+	+
4	4.85 ± 0.06	+ +	+	+	+	+	+
			Salinized				
1	5.07 ± 0.03	+	+ +	+	+ +	+ +	+
2	5.36 ± 0.02	+	+ +	+	+ +	+ +	+
3	5.95 ± 0.09	+	+ +	+	+ +	+ +	+
4	6.38 ± 0.06	+	+ +	+	+ +	+ +	+

— = absent; Tr = Trace; + = present; + + = moderate

Table 3. Effect of NaCl salinity on cuticular transpiration CCT in pigeon pea and gingelley (CT, mg H_2O cm^{-2} h^{-1}).

Stage	Pigeon pea		Gingelley	
	Control	Salinized	Control	Salinized
1	0.547 ± 0.062	0.327 ±0.027	0.568 ±0.027	0.404 ±0.074
2	0.513 ± 0.016	0.314 ±0.049	0.547 ±0.019	0.398 ±0.043
3	0.502 ± 0.024	0.298 ±0.043	0.532 ±0.028	0.362 ±0.042
4	0.498 ± 0.029	0.285 ±0.062	0.524 ±0.014	0.360 ±0.076

CT obtained when EW was partially removed (3, 8). Some recent reports also showed a close relationship between the chemical composition of wax and CT (1, 6), which indicated that primary and secondary alcohols and aldehydes play a significant role in reducing cuticular transpiration. Similarly, in the present study a reduced CT is associated with with a linear relationship between CT and 1/amount of EW (2). The results of the present study showed a similar trend, as shown in Figure 1. The importance of EW for CT has been indicated by the increase in high amounts of alcohols and aldehydes in the EW of pigeon pea and gingelley under salinity (Tables 1 and 2).

It is concluded that accumulation of high amounts of epicuticular wax causing an adaptive reduction in cuticular transpiration as observed in the present study, might be an adaptive feature in addition to the development of thick cuticle and induction of succulence (5, 7). The wax layer thus plays an important role in minimising water loss, so that the plants can cope with the adverse conditions created by salinity.

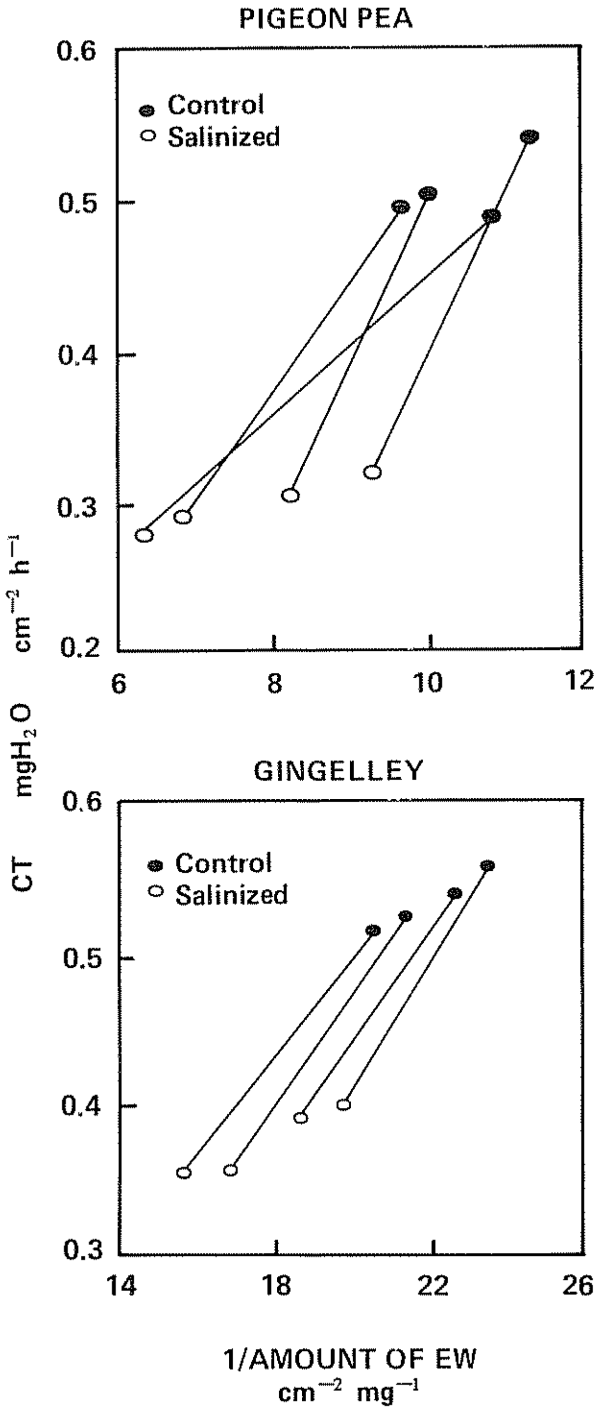

Fig. 1. Relationship between cuticular transpiration, CT and epicuticular wax, EW in pigeon pea and gingelley under NaCl salinity.

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