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## Salt tolerance evaluation in guava germplasm

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**Abstract** The five-month-old plants obtained from stem-cutting of 20 guava genotypes were grown in sand culture with 200 mM NaCl for 19 days to evaluate their salt tolerance. The primary symptom of salt stress, leaf burn, appeared from the young to the mature and from the margin to the center of leaves in all genotypes were reported. Based on leaf burn severity, the 20 guava genotypes were separated into four groups by level of tolerance. One genotype (KUHP38) was highly tolerant, three genotypes ('Paen Seethong', 'Na Suan', and KUHP12) were moderately tolerant, eight genotypes were sensitive, and eight genotypes were highly sensitive to salt stress. The highly tolerant genotype showed high survival and slight leaf burn, while the highly sensitive genotypes showed severe leaf burn and even death of some plants. Sodium and chloride content in the leaves, stems, and roots of the highly sensitive genotype was higher than in the highly tolerant genotype. Based on the results obtained, 'Paen Seethong' and 'Na Suan' were recommended for desert-type commercial plantings in the saline soil regions of Thailand, while KUHP38 and KUHP12 may be used as rootstocks. These genotypes could be used as breeding materials for developing new cultivars with high salt tolerance.

**Keywords:** Fruit breeding, guava breeding, germplasm evaluation, NaCl, *Psidium guajava* L.

### Introduction

One hundred million hectares (ha), or 5% of the world's cultivated lands, are adversely affected by high salt concentration (Ghassemi *et al.*, 1995). In Thailand, especially the central and northeast parts of the country, an important limitation of agricultural production is salinity, mainly due to NaCl salt accumulation (Im-Erb *et al.*, 2013). Salinity reduces crop yield, transpiration, and enzymatic activities due to osmotic effects (water stress), specific ion toxicities, and habitat effects (deflocculating of soil clay particles and loss of air-filled pore space) (Orcutt and Nilsen, 2000). Consequently, experiments in pear (Okubo and Sakuratani, 2000), citrus (Ruiz *et al.*, 1999), and guava (Ebert *et al.*, 2002) have been designed to understand the physiology of salt stress in order to determine the best cultural practices for managing salt problems in the field.

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Another approach to this problem is the selection of crop genotypes with high levels of tolerance to salt stress, the model we have adopted for this paper.

Guava (*Psidium guajava* L.) is an important tropical fruit in Thailand. The guava is regarded as moderately salt-tolerant (Maas, 1993), with a salinity limit for successful guava production of 8 to 9 dS.m<sup>-1</sup> electrical conductivity of the soil saturation extract. Thus far, the cultivar has been reported to have good tolerance to highly saline conditions (>16 dS.m<sup>-1</sup>). However, more than 90 guava accessions have been collected from various countries by the guava breeding program of the Department of Horticulture at Kasetsart University, Khamphaeng Saen Campus in Nakhon Pathom, Thailand since 1998. These guava accessions vary widely in their fruit qualities (Thaipong and Boonprakob, 2006) and plant growth types (Thaipong *et al.*, 2017), but the salt tolerance of these accessions has not been quantified. The objective of the present experiment was to identify guava genotypes with high salinity tolerance.

## **Materials and Methods**

### ***Experimental materials***

The experiment was run during the months of July and August in a greenhouse at Kasetsart University, Kamphaeng Saen Campus in Nakhon Pathom, Thailand. Twenty guava genotypes (Table 1) were propagated from stem cuttings, placed in plastic pots (diameter of 8 inches) with 5 kg of sand, and supplied with 500 ml of a soluble fertilizer (20N-20P-20K) in 200 ppm, plus trace elements every week for five months. The five-month-old plants were treated with 500 ml of 200 mM NaCl, plus 200 ppm of the fertilizer every two days for 19 days. The NaCl concentration used in the experiment was selected from an our previous experiment involving 0, 100, 200, 300, and 400 mM NaCl. The results showed that 50% leaf burn was induced by 200 mM NaCl after 19 days of application and all plants survived, while the next two higher levels caused the plants to die (unpublished data). The surplus solution drained naturally from the bottom of the pots to avoid salt build-up in the growth media. The plants were sprayed daily with macro and micronutrients to avoid reactions of the salt and nutrient ions in the solution.

### ***Data collection***

Leaf burn (%) and the progression of leaf burn were recorded every two days. At the end of the experiment, the 20 cultivars were classified by salt tolerance level based on leaf burn (%) into the following categories: highly tolerant ( $\leq 20\%$ ), moderately tolerant (21-39%), sensitive (40-69%), or highly

sensitive ( $\geq 70\%$ ) to salt stress. Plants of one genotype from the highly tolerant and highly sensitive groups were separated into leaves, stems, and roots. Powdered samples of the oven-dried materials were analyzed for chloride and sodium concentration. Chloride was estimated using silver ion titration with a chloride meter (6610, Eppendorf, Germany). Sodium concentration was determined using an atomic absorption spectrophotometer (905AA, GBC, Australia).

## Results

### *Leaf burn symptom*

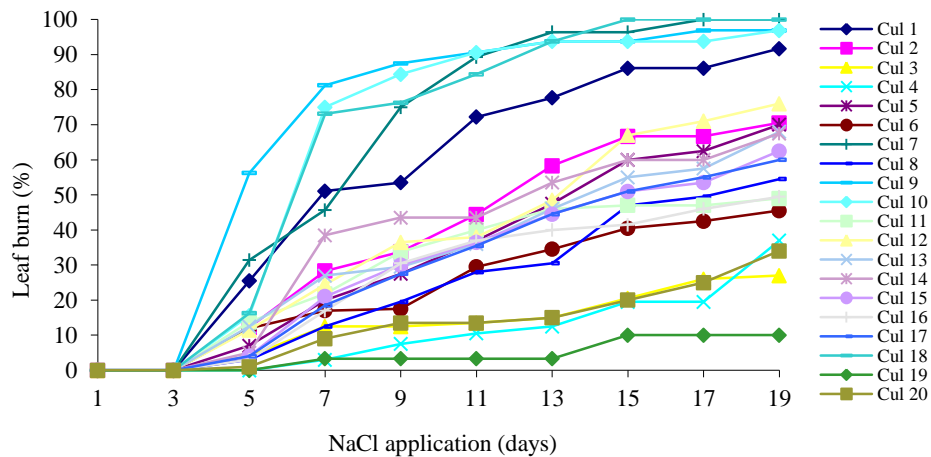
Guava plants treated with 200 mM NaCl showed the initial symptoms of leaf burn on young leaves on the fifth day of treatment (Figure 1). The leaf burn extended from the top to the bottom of the leaves, from the young to the mature, and from the margins to the center of the leaves. Leaf damage increased dramatically with the length of NaCl treatment (Figure 1). Severely burned leaves dropped. The salt stress plants also showed leaf yellowing. Based on leaf burn rating, the 20 genotypes can be classified into four salt tolerance groups: highly tolerant, moderately tolerant, sensitive, and highly sensitive (Table 1).

The one genotype of the highly tolerant group was KUHP38, which was introduced from Japan. KUHP38 began to show leaf burn after seven days of treatment, and the symptom developed slowly (Figure 1). Nineteen days after the first application, only 10% leaf burn was observed on this genotype (Table 1).

The three genotypes in the moderately tolerant group included 'Paen Seethong', which is a popular dessert type in Thailand; 'Na Suan', also a dessert type in Thailand; and KUHP12, which was introduced from Myanmar. 'Paen Seethong' began to show leaf burn after seven days of treatment, while the other two types showed the symptom within five days (Figure 1). Nineteen days after the first application, 37%, 27%, and 34% leaf burn rates were observed on the genotypes 'Paen Seethong', 'Na Suan', and KUHP12, respectively (Table 1).

Eight genotypes were in sensitive group: 'Pijit 13-10', KUHP10, KUHP21, KUPH30, KUHP33, KUHP34, KUHP35, and KUHP36. These genotypes were heterogeneous, with their leaf burn severity ranging from 46% for genotype KUHP21 to 68% for genotype KUHP36 (Table 1).

The other eight genotypes ('Klom Salee', 'Yen Song', 'Khoa Um-porn', 'Daeng Siam', KUHP16, KUHP20, KUHP29, and KUHP31) were highly sensitive to salt stress (Table 1). After 19 days of salt stress treatment, these plants grew weakly and showed burns on more than 70% of their leaf areas, and some plants died.



**Figure 1.** The progression of leaf burn (%) of 20 guava genotypes

**Table 1.** Leaf burn (%) of 20 guava genotypes after 19 days of NaCl application

Genotype	Utility/Type	Origin	Leaf burn (%)	Salt tolerance level
1. Klom Salee	Dessert/Commercial	Thailand	92 abc	Highly sensitive
2. Khoa Um-porn	Dessert/Commercial	Thailand	71 a-e	Highly sensitive
3. Na Suan	Dessert/Commercial	Thailand	27 gh	Moderately tolerant
4. Paen Seethong	Dessert/Commercial	Thailand	37 e-h	Moderately tolerant
5. Yen Song	Dessert/Commercial	Thailand	70 a-e	Highly sensitive
6. KUHP21	Dessert/Native	Thailand	46 e-g	Sensitive
7. KUHP29	Dessert/Native	Thailand	100 a	Highly sensitive
8. KUHP30	Dessert/Native	Thailand	55 d-g	Sensitive
9. KUHP20	Dessert/Native	Thailand	97 ab	Highly sensitive
10. Daeng Siam	Dessert/Commercial	Thailand	97 ab	Highly sensitive
11. KUHP10	Dessert/Native	Thailand	49 d-g	Sensitive
12. KUHP31	Processing/Advanced selection	Thailand	76 a-d	Highly sensitive
13. KUHP36	Processing/Advanced selection	Thailand	68 a-f	Sensitive
14. KUHP33	Processing/Advanced selection	Thailand	68 a-f	Sensitive
15. Pijit 13-30	Processing/Advanced selection	Thailand	63 b-f	Sensitive
16. KUHP35	Processing/Advanced selection	Thailand	50 d-g	Sensitive
17. KUHP34	Processing/Advanced selection	Thailand	60 c-g	Sensitive
18. KUHP16	Dessert/Native	Brazil	100 a	Highly sensitive
19. KUHP38	Dessert/Native	Japan	10 h	Highly tolerant
20. KUHP12	Dessert/Native	Myanmar	34 fgh	Moderately tolerant
			<i>P</i> value	< 0.01

Remark: The same letters within a column are not significantly different by Duncan's New Multiple Range Test following ANOVA ( $P < 0.05$ ).

### ***Ion accumulation***

Sodium and chloride concentrations in the leaves, stems (main stem plus branches), and roots at harvest (day 19) of the highly sensitive genotype were both greater than the sodium and chloride concentrations of the highly tolerant genotype (Table 2). In the case of the highly tolerant genotype, the highest sodium and chloride concentrations were found in the roots (Table 2). The highest Na<sup>+</sup> and Cl<sup>-</sup> concentrations of the highly sensitive genotype were observed in the leaves (Table 2), which were severely burned.

**Table 2.** Chloride and sodium concentration in the leaves, stems, and roots of cultivars highly sensitive and highly tolerant to salt stress

Plant part	Na (%)		Cl (%)	
	Highly sensitive	Highly tolerant	Highly sensitive	Highly tolerant
Leaves	3.79	1.31	6.35	2.15
Stems	2.81	0.58	3.96	0.70
Roots	3.08	2.26	3.28	2.45

### **Discussion**

In general, salt toxicity symptoms, especially leaf burn, progress differently among plant species. Ruiz *et al.* (1999) reported that NaCl produced irregular necrotic spots in the interval regions and necrosis along the margins and tips of the citrus leaves. Interestingly, Ebert *et al.* (2002) found that guava seedlings treated with 60 mM NaCl showed the first symptom of salt stress as leaf burn on the old leaves, but not on the young leaves as found in this study. This difference may be due to the concentration of NaCl used. The 200 mM NaCl concentration used in our research was very high and probably caused osmotic stress, which would decrease the plants' water absorption capability. Because young leaves are more sensitive to water deficits than old leaves, the first symptoms seen on young leaves in this experiment are possibly attributable to the effects of water stress rather than the toxicity of Na<sup>+</sup> or Cl<sup>-</sup>. Severely burned leaves dropped. Leaf drop might be a natural mechanism of survival that helps plants maintain their health. The salt stress plants also showed leaf yellowing, which has been attributed to a nutrient imbalance caused by salt stress (Orcutt and Nilsen, 2000). In this case, leaf yellowing may be the result of an N deficiency (Ebert *et al.*, 2002).

Sodium and chloride concentrations in the leaves, stems, and roots of the highly sensitive genotype were both greater than the sodium and chloride concentrations of the highly tolerant genotype (Table 2). This indicates that the highly tolerant genotype might have some specific mechanism for salt tolerance

(such as salt exclusion) to minimize the accumulation of these ions in the plant tissue (Orcutt and Nilsen, 2000).

## Conclusion

The tolerant genotypes, 'Paen Seethong', 'Na Suan', KUHP38, and KUHP12, can be recommended for commercial planting or rootstocks for other commercial cultivars in the saline soil regions of Central and Northeast Thailand. Furthermore, these cultivars are good materials for breeding new guava cultivars with high salinity tolerance.

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