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Selection of Rootstocks for Flooding and Drought Tolerance in Citrus Species

Ram Chandra Bhusal, Fusao Mizutani and Kipkoriony Laban Rutto
The Experimental Farm, Faculty of Agriculture, Ehime University,
Hojo, Ehime Prefecture, 799-2424, Japan

Abstract: The tolerance levels of citrus trees to different degrees of drought and flooding was studied by using twenty-three species and varieties. Rough lemon, trifoliate orange, lemon and Cleopatra mandarin were evaluated as the most flooding tolerant; Yuzu, 'Kawano' natsudaidai, Kinukawa-mikan, 'Tanaka' navel orange and Ponkan as medium; and Hana-daidai, Kosuito, Naruto-mikan and Natsudaidai as least tolerant. Trifoliate orange, Yuzu, Shiikuwasha, Hana-daidai, 'Tanaka' navel orange, Natsudaidai, Shuto, Yamabuki , Ponkan and 'Shiranui' were evaluated as the most drought tolerant; Cleopatra mandarin, Naruto-mikan and Murcot as medium and Rough lemon, lemon, Ban-okan and 'Kawano' natsudaidai as drought intolerant. The effect of flooding and drought on 1-aminocyclopropane-1-carboxylic acid (ACC) content and ethylene production in the roots of these several species and varieties was determined. The ACC content and ethylene production in the roots increased more rapidly in sensitive species in response to water stress. Under flooding conditions, roots maintained higher ethylene production levels than control roots. Sensitive species showed a little greater levels in ethylene production than the tolerant ones.

Key words: Citrus species, flooding, drought, trifoliate orange, Yuzu, ethylene

Introduction

Generally crop plants require well drained soils for maximum growth and productivity. Perennial crops such as fruit trees are particularly vulnerable, since poor drainage, even for a short period in one season, can have long term effects on productivity (Woudt and Hagan, 1957). Morphological alternations such as formation of adventitious roots and aerenchyma have frequently been observed in flooded roots (Blom and Voesenek, 1996; Kludze *et al.*, 1994). In the shoots, changes in water relations occur and a decrease in stomatal conductance can be caused by reductions in the soil-plant hydraulic conductance (Vartapetian and Jackson, 1997). Waterlogging also influences nutrient uptake by both woody species and herbaceous plants. The effect of waterlogging is more severe during the growing season than when the trees are dormant (Salesses *et al.*, 1970). There is a differential sensitivity of fruit tree species to waterlogging. Citrus are classified as an intermediate group (Rowe and Beardsell, 1973).

On the other hand, over half the world's land surface is exposed to periodic drought (Boyer, 1982) and drought condition also equally disturb plant growth. Drought affects many physiological processes of most plants including citrus (Kramer, 1983). The effects of water stress depend on the timing, duration and magnitude of the deficit (Bradford and Hsiao, 1982). Stomatal closure is a passive response to water deficit, and it may be related to hormonal changes (Mansfield, 1987; Davies and Zhang, 1991). Citrus trees can neither grow nor fruit well without frequent and timely irrigation throughout their life. Citrus trees require more water sap circulation than deciduous trees of equal age and size in which transpiration is greatly reduced during dormancy and they come under species having low drought tolerance (Hurvitz, 1958). When drought condition continues, the stomata progressively lose their ability to close and finally remain permanently open and the plants defoliate, wilt and die. Flooding and drought cause hypoxia and water deficit, respectively and affect plant activities such as photosynthesis, transpiration, evaporation and translocation in citrus. Plants cannot continue their growth process without water and oxygen although plants species have different capacities for tolerance. In this condition, a better understanding of water tolerance and drought tolerance of citrus species and varieties and their suitability as rootstocks would help in improvement of the citrus industry under various climates. The objective of this study was to examine the tolerance levels of several citrus species and varieties to different degrees of drought and flooding.

Materials and Methods

The experiment was conducted at the University Farm, Ehime University, Japan during July 1999 to March 2001. This work consisted of two experiments. The 23 citrus species and varieties used in the first experiment are shown in Table 1. In the first experiment, the 23 citrus species and varieties were included in a flooding and drought exposure experiment. All species used in this experiment were one-year old. They were propagated by stem cuttings to perpetuate the characteristics of the parent plant and to maintain uniformity. The flooding experiment was conducted as 50 and 100% flooding with a non-flooded control. Each control experiment pot was irrigated with 250 ml water every day. In 50 and 100% flooding, treatment pots were placed in 90 x 60 cm^2 plastic containers. Containers were filled with tap water and water level was maintained to cover 50 and 100% pot soil, respectively. Water level was checked daily during the experiment period lasting from July 1 to September 1, 1999. In this period all treatments were visually evaluated and 0-5 score was given at weekly intervals. The score was given on the basis of yellowing, defoliation and wilting. Data was recorded as a minimum of 0 for normal plants and a maximum of 5 for extremely injured plants. A final score was given on September 1, 1999 after two months of continuous flooding.

Table 1: Citrus species and varieties used in the first experiment.

S No.	Botanical names	Common names
1.	*Citrus jambhiri* Lush.	Rough lemon
2.	*Poncirus trifoliata* [L.] Raf.	Trifoliate orange
3.	*Citrus limon* [L.] Burm. f.	Lemon
4.	*Citrus reshni* hort. ex Tanaka	Cleopatra mandarin
5.	*Citrus junos* hort. ex Tanaka	Yuzu
6.	*Citrus grandis* [L.] Osbeck	Ban-okan
7.	*Citrus natsudaidai* Hayata	'Kawano' natsudaidai
8.	*Citrus sinensis* Osbeck	Kosuito
9.	*Citrus depressa* Hayata	Shiikuwasha
10.	*Citrus glaberrima* Tanaka	Kinukawa-mikan
11.	*Citrus medioglobosa* hort. ex Tanaka	Kikudaidai
12.	*Citrus medioglobosa* hort. ex Tanaka	Naruto-mikan
13.	*Citrus aurantium* L.	Hana-daidai
14.	*Citrus murcot* Smith	Murcot
15.	*Citrus sudachi* hort. ex Shirai	Sudachi
16.	*Citrus sinensis* Osbeck	'Tanaka' navel orange
17.	*Citrus iyo* hort. ex Tanaka	'Miyauchi' iyo
18.	*Citrus iyo* hort. ex Tanaka	'Katsuyama' iyo
19.	*Citrus natsudaidai* Hayata	Natsudaidai
20.	*Citrus aurantium* L.	Shuto
21.	*Citrus yamabuki* hort. ex Y. Tanaka	Yamabuki
22.	*Citrus reticulata* Blanco	Ponkan
23.	(*C. unshiu* Marc. x *C. sinensis* Osbeck) x *C. reticulata* Blanco	'Shiranui'

The drought experiment was based on 3, 7 and 14-days irrigation intervals. The drought experiment also started on July 1, 1999 and visual evaluation was done at weekly intervals for two months. The control plants were the same for flooding and drought treatments. Plants were irrigated with 250 ml of water per pot at intervals of 3, 7 and 14 days corresponding to the three treatments. The same procedures mentioned in flooding treatment were used for visual evaluation and data recording. On the basis of visual evaluation and data analysis, the 23 citrus species were divided into tolerant, moderately tolerant and sensitive groups with regard to flooding and drought tolerance.

In second experiment, among different species trifoliate orange and Yuzu were selected as the most and moderately resistant to flooding respectively and trifoliate orange and Cleopatra mandarin as a similar order to drought. Trifoliate orange and Yuzu were flooded and trifoliate orange and Cleopatra mandarin were water-stressed. Flooding and drought were continued up to 14 days and fine root samples were collected on 0, 3, 7 and 14 days after the start of experiment for ACC and ethylene analysis.

The fine root samples (500 mg) were collected and were put into 20 ml plastic syringe and capped with rubber septum. After incubation for 4 hours at 25 ° C, a 1 ml gas sample was taken from the syringe and ethylene was analyzed by FID-GC with an activated alumina column.

The fine root samples (500 mg) were collected and put in 5 ml of 80 % ethanol and stored in the freezer until analysis. Samples were homogenized with sea sand in 80% ethanol and filtered. The extract was then evaporated in vacue to dryness. Then the residue was dissolved in 4 ml distilled water. An aliquot of the solution was used for ACC determination by following the method of Concepcion *et al.* (1979).

Results and Discussion

In the 100% flooding treatment, there was a clear difference in injury between the tolerant and sensitive species and varieties after two months of flooding. Based on a flooding injury index, the 23 species and varieties were divided into three groups: tolerant (Group-A), moderately tolerant (Group-B) and sensitive (Group-C). Rough lemon, trifoliate orange, lemon and Cleopatra mandarin were evaluated as being the most tolerant species; Yuzu, 'Kawano' natsudaidai, Kinukawa-mikan, 'Tanaka' navel orange, Shuto and Ponkan as moderately tolerant; and Hana-daidai,

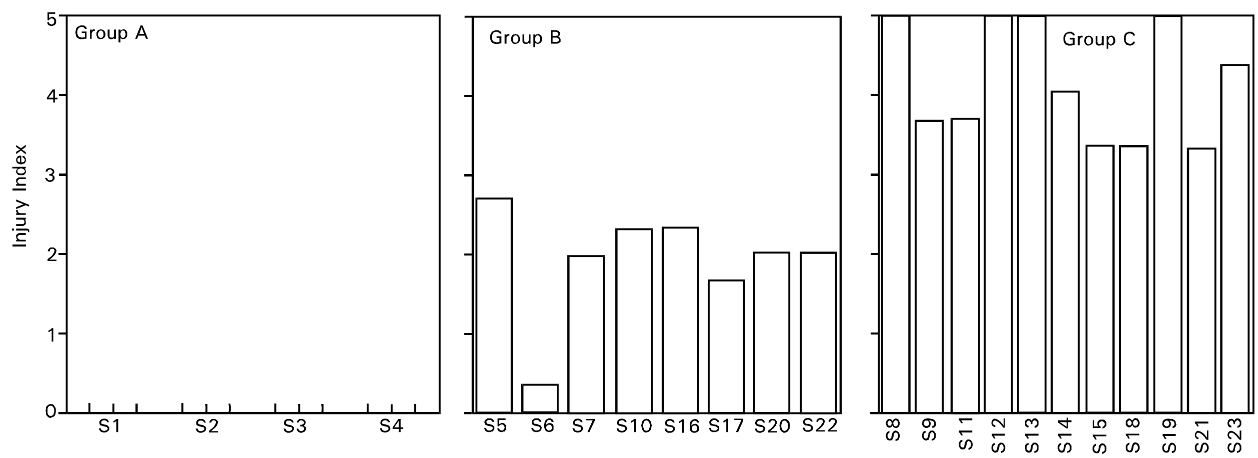

Fig. 1: Hundred % flooding injury index on September 1
Flooding injury was estimated from the following index (0= 0%, 1= 20%, 2= 40%, 3= 60%, 4= 80%, 5= 100%) representing the injury of plants after two months of flooding. Group A, B and C represent tolerant, moderately tolerant and sensitive species respectively. The numbers along the abscissa represent the following citrus species and varieties: 1. Rough lemon (*Citrus jambhiri* Lush.) 2. Trifoliate orange (*Poncirus trifoliata* [L.] Raf.) 3. Lemon (*Citrus limon* [L.] Burm. f.) 4. Cleopatra mandarin (*Citrus reshni* hort. ex Tanaka) 5. Yuzu (*Citrus junos* hort. ex Tanaka) 6. Ban-okan (*Citrus grandis* [L.] Osbeck) 7.'Kawano' natsudaidai (*Citrus natsudaidai* Hayata) 8. Kosuito (*Citrus sinensis* Osbeck) 9. Shiikuwasha (*Citrus depressa* Hayata) 10. Kinukawa-mikan (*Citrus glaberrima* Tanaka) 11. Kikudaidai (*Citrus canaliculata* hort. ex Y. Tanaka 12. Naruto-mikan (*Citrus medioglobosa* hort. ex Tanaka) 13. Hana-daidai (*Citrus aurantium* L.) 14. Murcot (*Citrus murcot* Smith) 15. Sudachi (*Citrus sudachi* hort. ex Shirai) 16. 'Tanaka' navel orange (*Citrus sinensis* Osbeck) 17.'Miyauchi' iyo (*Citrus iyo* hort. ex Tanaka)18.'Katsuyama' iyo (*Citrus* iyo hort. ex Tanaka) 19. Natsudaidai (*Citrus natsudaidai* Hayata) 20. Shuto (*Citrus aurantium* L.) 21. Yamabuki (*Citrus yamabuki* hort. ex Y. Tanaka) 22. Ponkan (*Citrus reticulata* Blanco) 23.'Shiranui' ([*C. unshiu* Marc. x *C. sinensis* Osbeck] x *C. reticulata* Blanco)

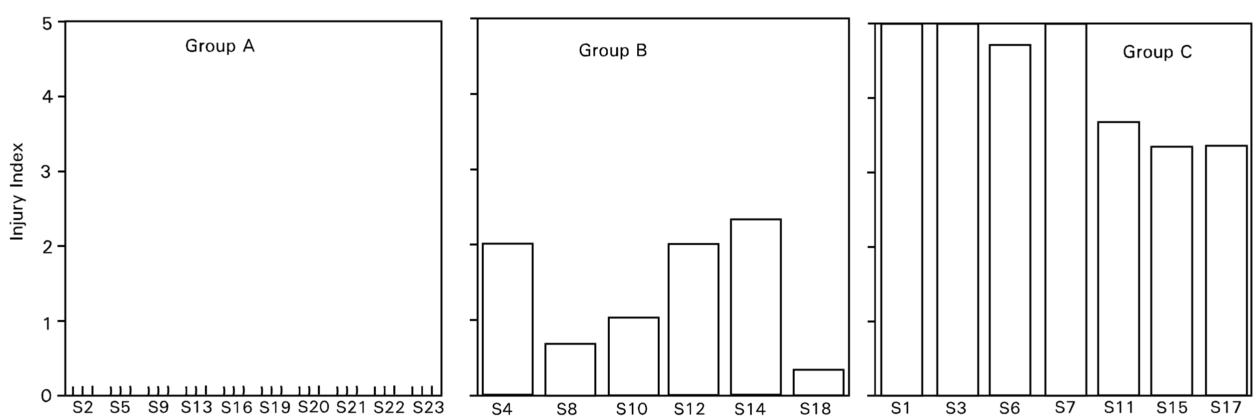

Fig. 2: Seven days interval irrigation injury index on September 1
Drought injury was estimated from the following index (0= 0%, 1= 20%, 2= 40%, 3= 60%, 4= 80%, 5= 100%) representing the injury of plants after two months of drought. Group A, B and C represent tolerant, moderately tolerant and sensitive species respectively. For the numbers along the abscissa, see the caption in Fig. 1.

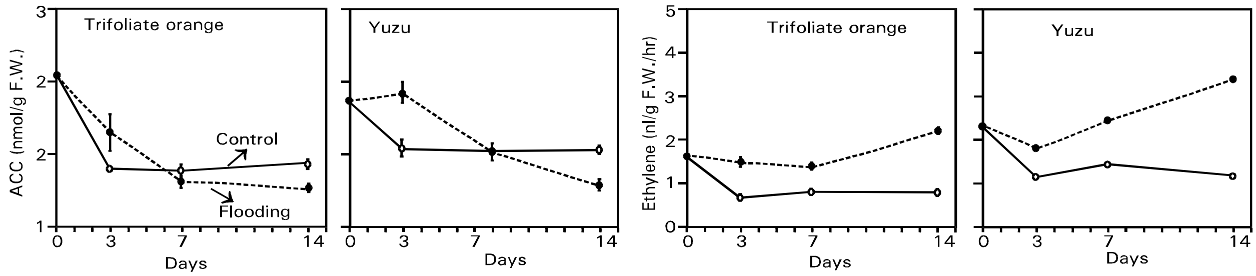

Fig. 3: ACC content and ethylene production in trifoliate orange and Yuzu roots under flooding. Bars in the figure represent means ± standard errors (SE)

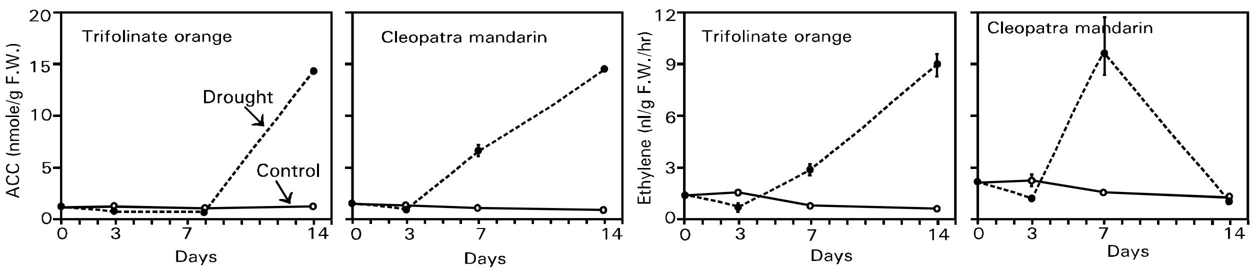

Fig. 4: ACC content and ethylene production in trifoliate orange and Cleopatra mandarin roots under drought. Bars in the figure represent means ± standard errors (SE)

Kosuito, Naruto-mikan and Natsudaidai as least tolerant (Fig. 1).

In the drought experiment, all 23 species and varieties suffered drought stress in 14-days irrigation interval while only a few species and varieties were affected in the 3-days irrigation interval treatment. There was an apparent difference in injury between the tolerant and sensitive species and varieties in the 7-days irrigation interval. As in flooding experiment, the 23 species and varieties were similarly divided into three groups: tolerant (Group-A), moderately tolerant (Group-B), and sensitive (Group-C) based on a drought injury index. Trifoliate orange, Yuzu, Hana-daidai, 'Tanaka' navel orange, Natsudaidai, Shuto, Yamabuki, Ponkan and 'Shiranui' were evaluated as the most drought tolerant; Cleopatra mandarin, Naruto-mikan and Murcot as moderately tolerant and Rough lemon, lemon, Ban-ohkan and 'Kawano' natsudaidai as drought sensitive (Fig. 2).

The ACC levels were reduced in both flooded and control roots during the flooded periods but were maintained higher in the flooded roots after 3-days of flooding. After 14-days the levels became lower than control roots. On the other hand, ethylene production was consistently greater in the flooded roots and was a little greater in Yuzu than trifoliate orange (Fig. 3). Kawase (1972) and Smith and Russell (1969) found that flooding induced ethylene production in flooded soils, submerged plant parts and roots but Culbert and Ford (1972) showed that oxygen deficiency was relatively harmless to citrus roots during 7 days of flooding. In drought experiment, both trifoliate orange and Cleopatra mandarin significantly increased root ACC levels in response to water stress. Ethylene production in trifoliate orange in the drought treatment increased after three days of drought and continued up to 14 days. Cleopatra mandarin also similarly responded but ethylene production reached a maximum after seven days and then decreased to the control level after 14 days (Fig. 4). At this point, Cleopatra mandarin roots were already dead. Morita *et al.* (1952) concluded in his study that root growth was more seriously affected than top growth in flooding. Gomez-Cadenas *et al.* (1996) reported that ACC accumulation in roots was found only in water stressed plants and that when water stressed plants were rehydrated, ACC decreased in roots and increased in leaves. In our results ACC significantly increased after three days in Cleopatra mandarin roots and only after seven days in trifoliate orange roots. There was no such drastic changes in ACC content or ethylene production in either trifoliate orange or Yuzu roots when flooded for up to 14 days. Therefore, tolerant species and varieties seem to accumulate later and small amounts of ethylene than sensitive species and varieties in response to water stress and flooding. In conclusion, trifoliate orange is the most tolerant to both flooding and drought among the 23 citrus species and varieties.

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