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*Full Paper*

# Suitable criteria for drought-tolerant peach rootstocks grown in northern Thailand

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**Abstract:** Peach growing in rainfed areas in the highlands of northern Thailand is suffering from drought conditions, which are becoming increasingly severe every year. Drought tolerant rootstocks provide one option to alleviate this problem. Thus, this study aims to find some guides for selecting drought-tolerant peach rootstocks. The local peach variety 'Red Angkhang' and 3 new hybrid cultivars '42047T1', '43060T1' and '43087T2' were used in this study. Two-year-old peach seedlings of each cultivar were grown in pots and divided into 2 groups. The first group consisted of well-watered plants (100% of evapotranspiration) and the second group consisted of water-deficit plants which received only 30% of evapotranspiration for 5 weeks. After that, the water-deficit peach seedlings were re-watered in the same manner as the well-watered plants for 2 weeks. Water stress led to a decrease in growth in all cultivars. The water-deficit tolerance of Red Angkhang was comparable to that of the new hybrid 42047T1, but the two cultivars used different mechanisms: Red Angkhang responded to water deficit by increasing only the root dry weight while hybrid 42047T1 also accumulated sorbitol. The 43060T1 and 43087T2 hybrids were less tolerant to water deficit and responded by decreasing the root dry weight with no sorbitol accumulation. From this study, we suggest that root dry weight and sorbitol concentration can be used to screen drought tolerant rootstocks in peach in northern Thailand.

**Keywords:** peach rootstock, *Prunus persica*, drought tolerance, root growth, sorbitol accumulation

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## INTRODUCTION

Peach (*Prunus persica* (L.) Batsch) has been grown in the highlands of northern Thailand for many decades and some varieties have adapted to the local environment. However, the highland areas are now encountering drought conditions which may become more severe in the near future. The accumulation of solutes as a result of osmotic adjustment is one line of plant response to water stress. In Rosaceae fruit trees such as apple, pear and peach, sorbitol is an important translocated sugar and several studies have reported the relationship between concentration of sorbitol and stress tolerance [1, 2]. In *Prunus* spp., sorbitol is the main solute reported to accumulate during stress periods [3]. Kanayama et al. [4] found that sorbitol accumulation in apple, peach, Japanese pear and European pear is enhanced by stress. Soluble sugars have also been reported to be involved in stress tolerance in maize and wheat [5]. In apple trees (*Malus domestica*) the water deficit affects the production of roots in varying degrees depending on the rootstocks and it was suggested that drought tolerance can be determined by root dry matter production [6]. In Thailand the breeding programme for drought-tolerant peach rootstocks commenced in 2002 [7] and there are currently some promising new hybrid cultivars. However, their mechanisms of drought tolerance are not well understood. Since sorbitol, soluble sugars and the production of roots have been reported to be involved in stress in Rosaceae fruit trees, this study aims to investigate whether these traits can be used as guides for screening drought-tolerant peach rootstocks grown in northern Thailand. The findings from this study may be useful for a peach breeding programme.

## MATERIALS AND METHODS

### Plant Materials and Experimental Design

This study was conducted at the Royal Angkhang Agricultural Station, Chiang Mai province (19°5'4.633"N, 99°0'2.537"E). The experiment was designed as a 2x4 factorial using a completely randomised design with 2 levels of irrigation and 4 peach cultivars, namely Red Angkhang (local cultivar) and there new hybrid cultivars from the rootstock breeding programme of the Royal Project Foundation, viz. ‘43060T1’ (AK1-1-1-35 x Fla 84-18C), ‘42047T1’ (open pollination of AK1-1-14-35) and ‘43087T2’ (open pollination of AK 1-1-12-35). Two-year-old peach seedlings were planted in pots (15-inch diameter) with 2 replications (trees) per treatment and placed under a plastic roof. The field experiment started in April-May, 2011 (hot and dry season). The average temperature and relative humidity were 21$^{o}$ and 85% respectively. Before irrigation, the plants were weighed to determine the daily evapotranspiration for 2 weeks using a method modified from Rieger et al. [8]: the average daily evapotranspiration was found to be 1,100 mL/tree. Then the plants were divided into 2 groups. To each plant in the first group, the well-watered plants used as control group, 1,100 mL of water (100% of evapotranspiration) was applied daily, whereas each plant in the second group, the water-deficit plants, received a daily dose of only 330 mL (30% of evapotranspiration) for 5 weeks. After that, the water-deficit seedlings were watered everyday for 2 weeks with the same amount of water as the control seedlings.

### Sampling, Shoot and Root Variables

Sampling was taken at 3 stages: the first day and the last day of water-deficit treatment and 2 weeks after full watering. Two leaf discs (1 cm$^2$) were punched out from the fourth or the fifth leaf from the tip for sugar concentration determination.

In each plant, 4 shoots, each measuring 20 cm in length, were tagged and their increased length was monitored on the first and last day of water deficit and 2 weeks after full watering. Each plant was then collected and separated into shoot and root. The shoot was weighed, then kept at 80º for 72 hr or until dry and the dry mass was determined. The roots were washed and separated into coarse roots ($\geq$2 mm in diameter) and fine roots (<2 mm in diameter) before drying at 80º for 72 hr or until dry and the dry mass was determined.

## Sugar Analysis

Fresh leaf samples were used for determination of soluble sugars (sucrose, glucose and fructose) and sorbitol. Briefly, 0.05 g of each sample was cooled in liquid nitrogen and finely crushed in a precooled eppendorf tube with a small pestle. Then 1 ml of nanopure water was added and the sample was sonicated for 15 min. After centrifugation at 12,000 rpm for 15 min., the supernatant was collected, filtered through a 0.45-μm filter and stored at -20$^{o}$ for further analysis. The total sugars extracted from the sample were analysed by high-performance liquid chromatography using a Shimadzu chromatograph coupled with refractive index detector and a method modified from that of Karkacier et al. [10]. The analytical column was a CABOSep coregel-87C equipped with a guard column. The mobile phase was deionised water, the injection volume was 50 μL and the flow rate was 0.6 mL min$^{-1}$.

## Statistical Analysis

Analysis of variance (ANOVA) was performed to test for statistical differences among rootstocks and water treatment regimes. Cluster analysis was performed in order to group these rootstocks based on their drought response.

## RESULTS AND DISCUSSION

The shoot length, shoot fresh weight and shoot dry weight of water- deficit plants were less than those of the well-watered plants although the shoot dry weight shows no significant difference in all cultivars (Tables 1 and 2). Similar results were also reported for a *Eucalyptus* tree species [11] and Imperial Gala apple trees [12] when subjected to water stress.

At the end of week 5, water stress affected root growth in each cultivar, although in a different manner. The root dry weight of the water-deficit plants decreased in cultivars 43060T1 and 43087T2, whereas it increased in Red Angkhang and 42047T1. The coarse root weight in the well-watered plants increased slightly in Red Angkhang and 42047T1 while the fine root weight of 42047T1 and Red Angkhang increased by 50.20% and 1.89% respectively (Figure 1 and Table 2).

The study on drought tolerance of rootstocks has focused on the root system. Commercial apple rootstocks M26 and MM111 have been reported to be drought-tolerant rootstocks [9]. One suggestion is that drought tolerance, at least in part, is determined by root dry matter production [13], which is in agreement with the work of Atkinson et al. [6] with apple plants (*Malus domestica*). They used the new rootstock selections, AR295-6, AR360-19 and AR628-2, which produced fewer fine and coarse roots in response to water deficit, whereas in rootstocks AR69-7 and M26, root production increased. This suggests that water deficit influences the production of coarse and fine roots differently and that the response varies with rootstocks. An increase in root growth might produce a greater root surface area leading to an increase in water absorption. Since roots are the only means of acquiring water from the soil, their growth, density and size

**Table 1.** Shoot fresh weight and shoot dry weight in peach seedlings during water deficit and recovery periods

| Cultivar | Treatment | Shoot fresh weight (g) |  |  | Shoot dry weight (g) |  |  |
|---|---|---|---|---|---|---|---|
|  |  | Day beginning | Last day of 5th week | Last day of 2-week recovery | Day beginning | Last day of 5th week | Last day of 2-week recovery |
| **Red Angkhang** | Full watering | 210.0 | 300.1 | 456.0 | 99.6 | 134 | 215.2 |
|  | Water deficit |  | 291.6 | 240.9 |  | 129.3 | 129.9 |
| **43060 T1** | Full watering | 150.5 | 305.1 | 351.8 | 70.6 | 140.5 | 170.5 |
|  | Water deficit |  | 262.7 | 337.5 |  | 134.3 | 169 |
| **43087 T2** | Full watering | 204.5 | 320.3 | 425.0 | 95.9 | 154.7 | 206.4 |
|  | Water deficit |  | 249.4 | 264.0 |  | 141.8 | 139.9 |
| **42047 T1** | Full watering | 247.5 | 326.1 | 369.4 | 122.25 | 156.3 | 182.6 |
|  | Water deficit |  | 278.9 | 252.1 |  | 159 | 128.9 |
| **Significance:** |  |  |  |  |  |  |  |
| **Cultivar** |  | ns[1] | ns | ns | ns | ns | ns |
| **Water deficit** |  | - | * | * | - | ns | ns |
| **Interaction** |  | - | ns | ns | - | ns | ns |
| **CV** |  | 26.1 | 13.9 | 16.3 | 33.89 | 17.4 | 11.5 |

Note: ns = not significant; * indicates significant difference from control at $P \leq 0.05$; CV = coefficient of variation
[1] Between water treatments

**Table 2.** Increases in shoot length and root dry weight in peach seedlings during water deficit and recovery periods

| Cultivar | Treatment | Increased shoot length (cm) |  | Root dry weight (g) |  |  |
|---|---|---|---|---|---|---|
|  |  |  |  | After 5 weeks |  |  |
|  |  | week 0-5 | week 5-7 | Total Root | Tap Root | Fine Root |
| **Red Angkhang** | Full watering | 17 | 19.2 | 116.6 | 97.1 | 19.5 |
|  | Water deficit | 10.8 | 11.8 | 122.7 | 102.8 | 19.9 |
| **43060T1** | Full watering | 16.5 | 18.4 | 128.8 | 107.2 | 21.7 |
|  | Water deficit | 5.8 | 7.8 | 115.3 | 94.6 | 20.7 |
| **43087T2** | Full watering | 17.1 | 21.8 | 139.2 | 108.4 | 30.8 |
|  | Water deficit | 10.6 | 12.2 | 126.9 | 103.9 | 23 |
| **42047T1** | Full watering | 19.5 | 21.6 | 145.6 | 125.8 | 19.8 |
|  | Water deficit | 9.8 | 22.8 | 158.4 | 128.6 | 29.8 |
| **Significance:** |  |  |  |  |  |  |
| **Cultivar** |  | ns[1] | ns | ns | ns | ns |
| **Water deficit** |  | * | ns | ns | ns | ns |
| **Interaction** |  | ns | ns | ns | ns | ns |
| **CV** |  | 41.5 | 24.9 | 14.4 | 15.7 | 16.0 |

Note: ns = not significant; * indicates significant difference from control at $P \leq 0.05$; CV = coefficient of variation
[1] Between water treatments

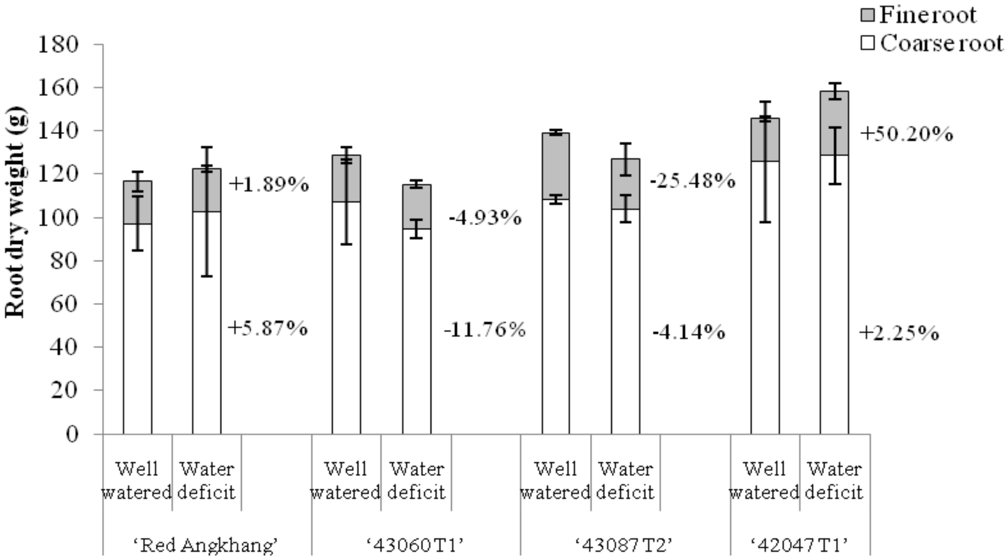

**Figure 1.** Dry weight of total roots, coarse roots and fine roots of well-watered and water-deficit peach seedlings after 5 weeks of water deficit

represent key responses by plants to drought stress. In our experiment, the increased root dry weight in Red Angkhang and 42047T1 may be considered a characteristic response to drought.

In Rosaceae fruit trees, many researches have reported the accumulation of sorbitol during drought stress [1, 14-16]. In the current study, before the water-deficit period, the sugar concentration was not significantly different among cultivars. However, at the end of the water deficit period, there were significant differences in the levels of soluble sugars, sorbitol and fructose among cultivars, but not after re-watering (Table 3). The increase in soluble sugars and fructose confirms earlier findings that soluble sugars accumulate in leaves during the drought stress of many fruit trees [14, 17, 18]. The highest accumulation of sorbitol was in the water-deficit 42047T1 cultivar, in which the sorbitol level increased significantly compared to other cultivars (Table 3). The accumulation of sorbitol differs depending on the degree of stress exposure or the genetic background [14, 19, 20]. The results in this study demonstrate that 42047T1 adjusts the osmotic pressure by accumulating sorbitol in order to maintain turgor when the plant encounters stress. This is in agreement with another work on peach [1], in which sorbitol was reported to accumulate in both the mature leaves and shoot tips of stressed plants from the second week of treatment, reaching up to 80% of the total solutes involved in osmotic adjustment. In the peach 'Ohatsumomo', sorbitol also accumulates in leaves during water stress [16], and in Japanese pear, the leaves respond to salt and low temperature stress by predominantly synthesising sorbitol [20]. Thus, the increase in the level of sorbitol in 42047T1 in response to water stress indicates that sorbitol plays a role in drought resistance in this cultivar.

**Table 3.** Soluble sugars (sucrose, fructose and glucose), sorbitol and fructose concentrations in peach seedlings during water- deficit and recovery periods

| Cultivar | Treatment | Soluble sugars (mg g$^{-1}$ fresh wt) | | | Fructose (mg g$^{-1}$ fresh wt) | | | Sorbitol (mg g$^{-1}$ fresh wt) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day beginning | Last day of 5th week | Last day of 2-week recovery | Day beginning | Last day of 5th week | Last day of 2-week recovery | Day beginning | Last day of 5th week | Last day of 2-week recovery |
| **Red Angkhang** | Full watering | 30.1 | 21.07 | 26.7 | 11.21 | 15.11 | 15.68 | 26.88 | 31.14 | 30.28 |
| | Water deficit | | 27.28 | 29.5 | | 17.7 | 20.9 | | 28.93 | 24.79 |
| **43060 T1** | Full watering | 25.55 | 25.6 | 20.89 | 7.21 | 18.47 | 12.15 | 23.15 | 29.26 | 25.97 |
| | Water deficit | | 28.99 | 23.87 | | 24.42 | 14.81 | | 38.45 | 28.27 |
| **43087 T2** | Full watering | 26.86 | 26.31 | 27.27 | 7.91 | 17.92 | 16.4 | 25.31 | 34.77 | 30.3 |
| | Water deficit | | 29.61 | 25.24 | | 22.19 | 18.47 | | 37.51 | 28.4 |
| **42047 T1** | Full watering | 41.1 | 34.65 | 29.26 | 14.61 | 25.79 | 20.28 | 28.02 | 35.88 | 31.8 |
| | Water deficit | | 43.31 | 27.07 | | 34.69 | 19.59 | | 48.18 | 28.03 |
| **Significance:** | | | | | | | | | | |
| **Cultivar** | | ns$^{1}$ | * | ns | ns | * | ns | ns | * | ns |
| **Water deficit** | | - | ns | ns | - | * | ns | - | * | ns |
| **Interaction** | | - | ns | ns | - | ns | ns | - | * | ns |
| **CV$^{2}$** | | 34.2 | 18.43 | 12.78 | 21.26 | 18.81 | 14.84 | 68.27 | 7.98 | 10.77 |

Note: ns = not significant; * indicates significant difference from control at $P \le 0.05$; CV = coefficient of variation

[1] Between water treatments

## CONCLUSIONS

By using the 8 parameters (shoot fresh weight, total root dry weight, coarse root dry weight, fine root dry weight, shoot length, soluble sugars, sorbitol and fructose) which show the most significant difference after water deficit for cluster analysis, the degree of drought tolerance of peach seedlings in this study can be separated into 2 groups. The first group has better tolerance under water deficit and consists of Red Angkhang, which increases its root dry weight, and cultivar 42047T1, which increases its root dry weight and also accumulates the highest amount of sorbitol. The second group consists of cultivars 43060T1 and 43087T2, whose the root dry weight decreases and there is no sorbitol accumulation. Compared with the local Red Angkhang cultivar, the new hybrid 42047T1 therefore appears to have better potential for drought tolerance than the other new hybrids 43060T1 and 43087T2.

We suggest that the root dry weight and sorbitol concentration can be used for screening drought tolerant peach rootstocks in northern Thailand.

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