Using *in vitro* technique for drought, heat, and combined drought-heat assay in potatoes

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Abstract. Drought and heat stresses are the significant abiotic stresses threatening the food crops, including potato. The in vitro technique is promising to conduct an abiotic stress assay. The assay of drought, heat, and combined drought-heat stress was conducted by using five potato lines. Polyethylene glycol (PEG) 8000 (25%) was used to induce drought stress. Heat stress was obtained by growing the plantlets at 35°C. Whereas placing the plantlets cultured in the media containing PEG 8000 (25%) at 35°C was applied to generate combined drought-heat stress. The results showed that four hours of abiotic treatments had no effect on growth of potato plantlets. The results showed that potato lines gave different responses to drought and combined drought-heat stress. Potato line 87HW13.7 wilted 8 hours after drought and combined drought-heat treatments, which became more severe by the time and died after 48 hours on combined drought-heat stress. Potato line 84.194.30 showed the wilting symptom after 24 hours due to drought stress and combined drought-heat stress and performed less wilting among five potato lines. The in vitro technique could differentiate the response of different potato lines to abiotic stress; therefore, this method would help the abiotic stress assay in potatoes.

1. Introduction

An environmental situation which unfavourable for plant growth and production is recognized as abiotic stress. Drought stress lead by water restriction and heat stress caused by high temperatures are the two most significant abiotic stresses concerning food production issue. Abiotic stress hampers crop productivity thus affects food security. The yield losses have been reported in a wide range of commodities [1,2].

Potato (*Solanum tuberosum* L.) is the third most important food crop in the world, based on consumption [3]. As the cool season crop, potato is sensitive to heat stress. This crop requires the optimum temperature at 24°C for plant growth and at 20°C for tuber yield [4]. Therefore, the temperature beyond those ranges could reduce plant growth and tuber production [5]. Potato is also known as a drought-sensitive plant since its root architecture, which is small and low [6]. Many attempts have been made to keep potato tuber production safe under abiotic stress conditions. These efforts involve culture techniques such as irrigation, mulching, and planting abiotic stress-tolerant varieties [7-10].
To cope with abiotic stress, developing abiotic stress-tolerant varieties is the most reliable way. However, the experiment in the factual field under abiotic stress circumstances would be hard to be practiced. An under-controlled environment has been used in various abiotic stress trials [11-13]. The *in vitro* condition could be an alternative for mimicking the field nature condition. Polyethylene glycol (PEG) is common inducing the drought since high-molecule weight could make osmotic stress by inhibiting water absorption from the media without transporting into the plant cells, non-toxic, and non-metabolized [14]. Whereas planting the materials in the growth chamber under high temperature could perform a heat stress experiment by adjusting other environmental factors, such as light and relative humidity [15]. Several *in vitro* experiments have reported the potato plant response to drought stress [12,13,16,17] and heat stress [15,18]. However, *in vitro* assay for drought-heat stress combination has not been adequately informed. This research aimed to investigate the effect of abiotic stresses, namely drought stress, heat stress, and combined drought-heat stresses, by using an *in vitro* assay in five potato lines.

2. Materials and method
This experiment was conducted in 2019 at Gene Research Center (GRC), University of Tsukuba, Japan, using factorial design in three replicates. The first factor was abiotic stress treatment, namely drought stress (D), heat stress (H), combined drought-heat stress (DH), and non-stress (C). Drought stress was induced by 25% Polyethylene glycol (PEG) 8000 [19]. Heat stress was conducted by incubating the plantlet at 35°C [15]. The combined drought-heat stress was created by placing the plantlets on 25% PEG8000 at 35°C. For non-stress conditions, plantlets were cultivated as in the initial incubation condition. The second factor was potato lines, covering 84.194.30 (L1), 86.61.26 (L2), 87HW13.7 (L3), DG-81.68 (L4), and Desiree (L5). Potato L1, L2, L3, and L4 are advance breeding line and supplied from Watanabe Lab, whereas Desiree is a commercial variety. With four first factors and five second factors, there were 20 combinations of treatments. Each treatment combination consisted of six test tubes and replicated three times. Firstly, potato plantlets were cultivated in the Murashige and Skoog (MS) medium [20] with 30 g l⁻¹ sugar, solidified with 1 % agar and cultivated for four weeks. Afterward, plantlets were transferred into test tubes contained liquid ½MS medium (half concentration of MS medium components) and cultivated for one week. These two steps were done in the culture room at 25 °C, relative humidity at 60% and photoperiod 16/8 light/dark. Abiotic stress treatment was applied on five weeks old plantlets. All plantlets were planted in the liquid ½MS medium.

Observation was made on wilting symptom, using wilting scoring: 1= no stress or all leaves turgescence; 3= 30% of the leaves are wilted; 5= 50% of the leaves are wilted; 7= 80% of the leaves wilted; 9= complete wilting [21]. Four hours after abiotic stress treatment, the wilting symptom was observed. This observation was also recorded at 8, 12, 24, and 48 hours after treatment. Analysis of variance (ANOVA) was carried out using R software, followed by Tukey’s HDS test at α = 0.05.

3. Results and discussion
During pre-treatment, *in vitro* plant materials showed good growth and performance. The first observation was done four hours after treatment. There was no single potato line shows the effect of abiotic stresses visually. Based on ANOVA results, abiotic stress, potato line, and the combination between abiotic stress and potato line were significantly affected the wilting symptom on potato *in vitro* plants at 8, 12, and 24 hours. ANOVA was not carried out at 48 hours of treatment since there were two potato lines died.

The mild wilting symptom showed at eight hours after treatment, only on potato 87HW13.7, under drought, and combined drought-heat stress (Figure 1A). It suggests that potato 87HW13.7 was the earliest sensitive to drought stress and combined drought-heat stress among the five potato lines tested. Twelve hours after treatment, except potato 84.194.30, all potato genotypes showed the wilting symptom due to drought stress and combined drought-heat stress (Figure 1B). This wilting became more severe 24 hours after treatment, and occurred in all potato lines, under drought and combined
drought-heat stress (Figure 1C). Until that time, heat stress did not induce wilting symptoms. The other symptoms were observed under heat stress, i.e., smaller and twisted leaves, and the leaf colour was brighter than under non-stress conditions. Heat stress disrupts cell membrane structure and composition which leads change of leaf size and form [22]. The bright colour might associate with chlorophyll. Reduction chlorophyll content under high temperature was reported in some species [23-25]. Observation on twelve hours after treatment seems to be the optimum time since after 48 hours of treatment, potato 87HW13.7 dan DG-81.68 could not survive under combined drought-heat stress, and totally wither under drought stress (Figure 1D).

From this experiment, heat stress treatment did not lead to withering. The mild symptom of wilting just observed after 48 h of heat treatment. It suggests that heat treatment in the in vitro condition does not induce water restriction in the short time of stress and does not change the plant water status. This situation might be explained that water content in the MS media did not evaporate much in the short time of heat exposure and can support plant growth. In maize, relative water content, which is an indicator of plant water status, decreases slightly due to heat stress but reduces significantly under drought and combined drought-heat stress [26]. However, the effect of heat stress in the in vitro condition in the extended time, need to be considered, by including more abiotic stress indicators.

Figure 1. Wilting score of five potato lines under various abiotic stress at different times (L3 and L4 died after 48 hours of DH treatment). ANOVA was not carried out at 48 hours treatment. Different letters above error bars indicate significant differences among treatments by Tukey’s HDS test at $\alpha = 0.05$. Error bars: standard error of mean. C: non-stress, D: drought stress, H: heat stress, DH: combined drought-heat stress, L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG-81.68, and L5: Desiree.

In the present study, the variation of wither responses by the time among different potato lines was observed under both drought stress (Figure 2A) and combined drought-heat stress (Figure 2B). Potato 87HW13.7 response was the fastest to either drought or combined drought-heat stress. After 8 hours of treatment, the wilting symptom was observed in potato 87HW13.7, with the wilting score was 2 and 2.33, under drought and combined drought-heat stress, respectively. At that time, the other potato lines were fresh and turgescence. By the time of treatment, potato 87HW13.7 and DG-81.68 became more
affected by drought and combined drought-heat stress. These two potato lines could not survive after 48 hours under combined drought-heat stress (Figure 2B). On the other hand, potato 84.194.30 was not affected by drought and combined drought-heat stresses for up to 12 hours of treatment. Even though, 24 hours after exposed to drought and combined drought-heat stresses, this line showed withering, as the other lines. The variation of potato lines response to single drought and heat stresses was reported by some research groups [27-29]. This variation shows the different of stress sensitivity among genotypes, indicates that every potato line has specific mechanism in response to abiotic stress. The response could be morphological, physiological, chemical, or molecular, which needs further investigation.

Figure 2. The increase of wilting score of five potato lines due to drought stress (A) and combined drought-heat stress (B) over time of treatment (L3 and L4 died 48 hours after combined drought-heat stress treatment). L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG-81.68, and L5: Desiree.

![Figure 2](image)

Figure 3. *In vitro* plants performance before abiotic treatment and after 24 hours of abiotic treatment.

C: non-stress, D: drought stress, H: heat stress, DH: combined drought-heat stress, L1: 84.194.30, L2: 86.61.26, L3: 87HW13.7, L4: DG-81.68, and L5: Desiree.

![Figure 3](image)

The wilting symptom is easy to be observed visually (Figure 3) and is common to be used in drought stress tolerance assay [30]. Generally, the wilting response of potato *in vitro* plant under
combined drought-heat stress was similar to that under drought stress, even stronger. Water deficit caused by drought stress leads to the cell turgor loss, then responded by wilting symptom [31]. Whereas during the period of experiment, in vitro potato plants did not give wilting respond to single heat stress. The in vitro technique for abiotic stress assay in potato needs a validation, since, in potato, the economic yield is the tuber, which is not covered by this method. This simple technique could be applied in a kind of evaluation that includes a vast amounts of accessions, such as germplasm screening or early selection for abiotic stress tolerance in potato breeding programs.

4. Conclusion
Response of in vitro potato plant to abiotic stress, particularly drought and combined drought-heat stress, was appeared visually as a wilting symptom. Abiotic stress assay by using the in vitro technique could detect the variation of wilting response among different potato lines. Based on wilting symptom, potato 84.194.30 was the most tolerant to drought stress and combined drought-heat stress. Combined drought-heat stress caused the most severe wilting symptom.

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