High temperature and water deficit are the most important abiotic stress that reduces the crop yield, and recently become more serious related to climate change (Lamaoui et al. 2018). In nature, heat stress and drought stress even co-occurs and results in unique plants response that different from each stress (Zandalinas et al. 2018). Crop loss due to combination drought and heat stress was reported bigger than single stress separately (Shah and Paulsen 2003, Dreesen et al. 2012). Plants develop various mechanisms and strategies to adapt to such conditions (Krannich et al. 2015). Information on the shift of phenotype in response to abiotic stress is crucial as the basis of maintaining the plant productivity; either, through the adaptation of cultivation techniques or developing abiotic stress-resistant cultivars. The shift of phenotype due to abiotic stress could be assessed directly on the plant, by which phenotyping, that could be performed by visual observation and simple measurement. Abiotic stress-related traits could help to evaluate the shift of phenotype due to environmental stress. The traits could be plant height, which is associated with growth form (Pérez-Harguindeguy et al. 2013); chlorophyll content, as one of important photosynthesis apparatus (Penuelas and Filella 1998); relative water content (RWC), that represents the balance of leaf tissue water supply and transpiration rate (Solts-Kalina et al. 2016); and cell membrane stability (CMS), since abiotic stress impairs cell membrane integrity (Blum and Ebercon 1981). The utilisation of these abiotic stress-related traits is common in abiotic stress evaluation, but only with single stress applied.
Potato (*Solanum tuberosum* L.), the third important main food crop in the world based on food supply (FAO 2019), is influenced by heat and drought stress adversely. Effect of drought stress and heat stress on potato plants were reported, from morphological, physiological, to molecular, but in individual stress (Iwama 2008, Dou et al. 2015, Rudack et al. 2017). The co-occurring drought and heat stress should get serious concern since this phenomenon is getting worse, as climate change causes an increase in the average earth surface temperature and low precipitation in some areas (Trenberth 2005, Andjelkovic 2018). However, information about the combination of drought and heat stress on potato plants is rare.

Evaluation of combined drought-heat stress on potato plants are then highly essential to get insight on potato plants’ response to the stress, whether the same with each stress separately or different from both. A controlled growth room experiment could be conducted for the seedling evaluation to eliminate the other factors than the primary abiotic stress-tested; moreover, it still represents the actual condition. The objectives of this experiment were to evaluate the morphological and physiological responses of different potato lines under drought and heat stresses and the combination of drought-heat stress.

**MATERIAL AND METHODS**

**Plant material and growing condition.** Four diploid potato breeding lines (L1: 84.194.30; L2: 86.61.26; L3: 87HW13.7, and L4: DG81-68) and one tetraploid potato cultivar Desiree provided from Gene and Research Center, University of Tsukuba, in term of *in vitro* plant, were used in this experiment. The *in vitro* plants were transferred into a growth room and transplanted in the plastic pot (diameter 10 cm) filled with granulated media: sterile peat moss:perlite: vermiculite (9:1:1:1). Shoot cuttings were done two weeks later, and the shoots were transplanted in cell tray using the same media. The three weeks of the rooted plant then transferred into quadrangular pots. The plants were grown under temperature 24 ± 1 °C, 40–60% relative humidity (RH), 12 h light/12 h dark. After ten days, abiotic stresses were given to ten plants each genotype in each replication, as follow:

- Non-stress (C): watered with Hoagland solution, temperature 24 ± 1 °C, 40–60% RH, 12 h light/12 h dark;
- PEG (polyethylene glycol)-induced drought stress (D): watered with Hoagland solution + PEG 8000 0.39 g/g H₂O (Michel 1983), temperature 24 ± 1 °C, 40–60% RH, 12 h light/12 h dark;
- heat stress (H): watered with Hoagland solution, temperature 30 ± 1 °C, 40–60% RH, 12 h light/12 h dark;
- combined drought-heat stress (DH): watered with Hoagland solution + PEG 8000 0.39 g/g H₂O, temperature 30 ± 1 °C, 40–60% RH, 12 h light/12 h dark.

The treatments were arranged in split plot design in six replications. Abiotic stress was used for the main plot, whereas the subplot was potato genotypes. This abiotic treatment was performed for 21 days, followed by recovery (as a non-stress condition) for 9 days.

**Plant growth.** Plant growth parameters included plant height (PH); leaf length (LL), and leaf width (LW). The relative growth rate of plant height ($RGR_{PH}$) was calculated followed (Chen et al. 2002):

\[
RGR_{PH} = \frac{\ln H_2 - \ln H_1}{T_2 - T_1}
\]  
(1)

where: $H_1$ – plant height at time 1 ($T_1$); $H_2$ – plant height at time 2 ($T_2$).

For $RGR_{PH}$ calculation, plant height measurement was done four times: before treatment (0 day treatment), middle of treatment (10 days treatment), end of treatment (21 days treatment), and after recovery (9 days recovery).

**Plant dry matter (PDM).** Measurement of PDM was done on three samples in each replication. Plant samples that free from media were weighed immediately for obtaining the fresh weight (FW). Then after oven-dried at 70 °C for 48 h, and weighed for the dry weight (DW). The PDM calculated using the formula:

\[
PDM (%) = \left(\frac{FW}{DW}\right) \times 100
\]  
(2)

**Wilting symptom (Wilt).** The wilting symptom was scored visually in three different times (middle of treatment, after treatment and after recovery) using the following score: 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 are wilted; 9 – complete wilted (Ekanayake 1989).

**Chlorophyll content (Chl) and photosystem II quantum yield (QY).** These two traits were measured at the same leaves sample from three plants in each replication. The chlorophyll content was measured by using SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan). Whereas QY was measured by
using FluorPen FP 100 (Photon Systems Instruments s.r.o., Drasov, Czech Republic).

**Cell membrane stability (CMS).** The sample for CMS assessment was harvested as the third or fourth leaf from the shoot from three plants in each replication. Cell membrane stability assessment was performed, followed by Rudack et al. (2017). The initial electrolyte leakage (C1) and final electrolyte leakage (C2) were measured by using Compact Conductivity meter B-771 (Horiba, Ltd., Kyoto, Japan). The value of CMS was calculated using the formula:

\[
CMS = \left(1 - \frac{C1}{C2}\right) \times 100
\]  

(3)

**Relative water content (RWC).** The leaf samples (3rd or 4th leaf from the shoot) were weighed immediately after harvested to get the fresh weight. Then the sample was soaked in the distilled water for 24 h and dried by blotting on a paper towel gently and weighed for the turgid weight (TW). The dry weight was measured after the leaf was dried in the drying oven for 24 h at 70 °C. The RWC was calculated using this formula:

\[
RWC (%) = \left(\frac{FW - DW}{TW - DW}\right) \times 100
\]  

(4)

**Stomatal traits.** Stomatal traits only observed on L5 (cv. Desiree) as the representative of common commercial potato cultivar in the middle of treatment. The observation was done by using Olympus System Microscope Model BX53 (Olympus Corporation, Tokyo, Japan) under 20× magnification, and measurement was made using the cellSens imaging software platform (Olympus Corporation, Tokyo, Japan). The stomatal trait that measured were stomata length (SL); stomata width (SW); stomata pore length (PL) and stomata pore width (PW).

All traits were measured from three different plants in each replication and performed at the end of treatment (except plant height, wilting symptom, and stomatal traits).

**Data analysis.** The analysis of variance (ANOVA) was done by using Agricolae Library in R version 3.4.4 (Mendiburu 2019). To illustrate the behavior changes of potato plants due to abiotic stress, response ratio (RR) was calculated as follow (Rymaszewski et al. 2017):

\[
RR = \ln\left(\frac{\text{mean value T}}{\text{mean value C}}\right)
\]  

(5)


**RESULTS AND DISCUSSION**

Analysis of variance showed that abiotic stress and genotype independently affected all traits (except QY), and the significant effect was demonstrated by their interaction on plant height, chlorophyll content, QY, and RWC (Table 1). Stomatal traits observed only in L5 (cv. Desiree) were also significantly affected by abiotic stress, including stomata length, stomata width, stomata pore length, and stomata pore width (data not shown).

The wilting symptom has been appeared in the middle of treatment under drought stress and combined drought-heat stress, and become more severe at the end of treatment (Figure 1). This symptom could be reduced in the recovery step by placing the plant in the non-stress condition. In the middle and end of treatment, combined drought-heat stress showed the highest symptom of wilting in all potato lines.

Wilting is a visual condition that easy to assess and commonly used to measure tolerance to drought stress (Engelbrecht et al. 2007). Water deficit causes

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**Table 1. Analysis of variance of abiotic stress-related traits**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Abiotic stress (F-value)</th>
<th>CVa</th>
<th>Potato line (F-value)</th>
<th>Abiotic stress × potato line (F-value)</th>
<th>CVb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height</td>
<td>28.33***</td>
<td>11.60</td>
<td>8.66***</td>
<td>3.09**</td>
<td>12.50</td>
</tr>
<tr>
<td>Leaf length</td>
<td>89.60***</td>
<td>11.50</td>
<td>10.47***</td>
<td>1.83</td>
<td>9.50</td>
</tr>
<tr>
<td>Leaf width</td>
<td>88.86***</td>
<td>12.80</td>
<td>5.73***</td>
<td>0.96</td>
<td>10.10</td>
</tr>
<tr>
<td>Plant dry matter</td>
<td>6.46***</td>
<td>16.10</td>
<td>12.08***</td>
<td>1.00</td>
<td>10.30</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>47.50***</td>
<td>9.80</td>
<td>26.77***</td>
<td>2.50**</td>
<td>10.40</td>
</tr>
<tr>
<td>Photosystem II quantum yield</td>
<td>0.61</td>
<td>5.00</td>
<td>2.70*</td>
<td>2.10*</td>
<td>3.10</td>
</tr>
<tr>
<td>Cell membrane stability</td>
<td>8.86**</td>
<td>7.80</td>
<td>7.91***</td>
<td>0.91</td>
<td>8.90</td>
</tr>
<tr>
<td>Relative water content</td>
<td>21.82***</td>
<td>12.70</td>
<td>6.33***</td>
<td>3.26**</td>
<td>7.20</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001; CVa – coefficient of variation of main plot; CVb – coefficient of variation of sub-plot and its’ interaction with main plot
decreasing leaf water potential that leads to reducing in leaf turgor potential and results in leaf wilting (Jensen 1981). In this case, the wilting symptom of potato plants under combined drought-heat stress is similar to that under drought stress.

The relative growth rate was calculated based on plant height. Drought stress suppressed the plant height faster than heat stress and combined drought-heat stress (Figure 2). However, in the extended treatment, combined drought-heat stress resulted in the slowest plant height growth rate in all potato lines. In this case, until the middle of treatment, $RGR_{PH}$ under combined drought-heat stress was similar to that under heat stress. Whereas, $RGR_{PH}$ after treatment was identical to under drought stress. Recovery in nine days after drought and combined drought-heat stress could increase the relative plant height growth rate in all potato lines except L2. Re-watering might...
help the plant to increase the turgor potential and recover the plant from the wilting symptom. The recovery capacity depends on genetic since the degree of recovery varies among the potato line.

All potato lines responded to the abiotic stress negatively by reducing the plant height, with exception L3 under heat stress (Figure 3A), and the leaf size in terms of leaf length (Figure 3B) and leaf width (Figure 3C) and combined drought-heat stress caused the highest reduction. These results revealed that combined drought-heat stress has the most negative effect on potato plant growth. Plant and plant organ’s

Figure 3. Potato plant traits respond to abiotic stresses as determined in the log response ratio. (A) Plant height; (B) leaf length; (C) leaf width; (D) plant dry matter; (E) chlorophyll content; (F) photosystem II quantum yield; (G) cell membrane stability, and (H) relative water content. D – drought stress; H – heat stress; DH – combined drought-heat stress; L1 – 84.194.30; L2 – 86.61.26; L3 – 87 HW 13.7; L4 – DG81-68; L5 – cv. Desiree
size is the result of cell numbers, cell size, and cell elongation (Bundy et al. 2012). Reducing the plant size, including leaf size, under abiotic stress conditions, is a result of lowering cell size since plant cells lose the turgor that required as a physical force for cell enlargement (Tardieu et al. 2014). In this case, the potato plant develops a stress tolerance strategy by maintaining cell turgor to reduce osmotic potential via reducing cell size (Zhang 2007).

Even though potato plant responses the abiotic stress by decreasing the plant height and leaf size, the opposite response in the plant dry matter content which generally increase, except L3 and L5 under heat stress (Figure 3D). Abiotic stress also known to impairs photosynthesize allocation to sink organ, which is tuber in potato plant. Therefore, the total photosynthesize then distributes mainly in the upper part of the plant, which has reduced by the abiotic stress effect.

The potato plants responded to abiotic stress positively by increasing chlorophyll content (Figure 3E), with an exception in three potato lines (L3, L4, and L5). Different from chlorophyll content, the response ratio of QY generally is negative (Figure 3F). However, heat stress was responded positively by L2 and L4, and combined drought-heat stress also was responded positively by L1 and L2. In this trait, L2 responded in a positive direction under all abiotic stress. The rising of chlorophyll content is one response mechanism under abiotic stress (Monneveux et al. 2014, Rolando et al. 2015). Leaf area reduction leads to the accumulation of chlorophyll in the leaf (Rolando et al. 2015). However, increasing the chlorophyll content did not follow by high QY. It is well-known that abiotic stress affects photosynthesis efficiency adversely by photo-inhibition phenomenon (Gururani et al. 2015). The photo-inhibition of PSII is indicated as a negative mechanism to protect PSII that results in a limit of photosynthesis process (Guidi et al. 2019). In this experiment, L2 increased the QY under heat and combined drought-heat stress, indicates that this line could adapt the stress until the period of measurement; however, it is unknown whether still high if the stress extended.

Potato plant responded to the abiotic stress negatively by decreasing CMS and RWC (Figure 3G, H). Drought stress and heat stress are known to disrupt the plasma membrane structure and composition, which consist of lipid and protein (Bray 1997, Asthir 2015). Electrolyte leakage measured from the membrane injuries caused by abiotic stress reflects the level of stability of membrane under abiotic stress (Arvin and Donnelly 2008). In this case, L1, L3, and L5 are more stable under drought, heat, and combined drought-heat stress since they have a small decrease in CMS. RWC is an indicator of plant water status that widely used in abiotic stress assessment (Soltyss-Kalina et al. 2016, Zhou et al. 2017, Tani et al. 2019). Different potato lines respond to the abiotic stress by decreasing their RWC at different levels, with the combined drought-heat stress produce the lowest RWC, suggested that the potato lines have different sensitivity to each abiotic stress. However, in general, all potato lines will be most sensitive when grown under combined drought-heat stress. In alfalfa, drought-tolerant genotypes show a low decrease of RWC (Tani et al. 2019). Three potato lines here (L1, L3, and L5) have a small reduction in RWC under all abiotic stress, suggested that they are tolerant of drought stress, heat stress, and combined drought-heat stress. This character implies the ability to maintain water status that crucial for the physiological process in the plant.

In the case of L5 (cv. Desiree), all abiotic stresses significantly decreased the size of stomata, in terms of stomatal length and stomata width; as well as stomatal pore length; however, in the stomata pore width, the decrease only observed in the drought stress (Figure 4). The response ratio to the abiotic stress of stomata length was similar to that of stomata width. A different response was performed on the stomata pore width, in which drought stress and combined drought-heat stress were responded positively by increasing the size of the stomata pore.
width (Figure 4). Stomatal closing and reducing stomatal size relates to turgor potential (Jensen 1981), and it is one osmotic stress avoidance strategy to minimise water loss via transpiration, which usually along with leaf size reduction (Zhang 2007). However, we should pay attention to the adverse effect that might be rise from the reducing plant height and leaf size to the tuber yield. Without a companion by high photosynthetic performance and assimilate transport, it will affect on tuber yield as potato tuber yield is a result of coordination between the source (leaves) and sink (tuber).

In general, potato plants respond to the unfavourable conditions caused by drought stress, heat stress, and combined drought-heat stress by suppressing their growth, in this case, by reducing the plant height and leaf size. Increasing the chlorophyll content under drought and combined drought-heat stresses did not accompany by the photosystem II quantum yield, which decreased in such conditions. Abiotic stresses also changed the relative water content and cell membrane stability in a negative direction. These abiotic stress-related traits were performed variously among the potato lines, indicates that there were different sensitivity in each potato line. Regarding the abiotic stress, generally, greater effects were produced under combined drought-heat stress. In further experiments, such information could be integrated into other study fields to get a more comprehensive solution for potato production under abiotic stress conditions.

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