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Comparison study of the physiology and simple phenolic content in cultivated and wild plants of *Paeonia lactiflora* pall.

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1 ABSTRACT
***Paeonia lactiflora* Pall. is included in the List of Very Rare Plants and Mongolian Red Book as very rare. The aim of research is to conduct a comparison study of water relation, chlorophyll fluorescence and the simple phenolic content in wild and cultivated plants of *P.lactiflora.* The water deficit was lower and water potential was higher in the cultivated (-0.577± 0.23 MPa) plant than in the wild plant (-1.403±0.41 MPa). The optimal quantum yield (QY) and ratio fluorescence decrease (Rfd) were higher (1.4-2.1 times) in the cultivated plant than in the wild plant. In addition, the chlorophyll index is higher in the cultivated plant (54.5±7.2) than in the wild plant (46.3±2.53). Our result showed that physiological process of cultivated plant is more active than the wild plant and the simple phenolic content was about 1 time higher in the leaves, stem, tuber of cultivated than in the wild plant. Eventually, it could be the optimal solution for conserving the natural wild plants from becoming extinct.**

2 INTRODUCTION
P. lactiflora was known as the white peony (*P. albiflora*) when first introduced into Europe. It was brought to England in the mid-18th century, and is the parent of most modern varieties. There are several hundred selected cultivars in a range of colours, sizes and forms; many have double flowers with the stamens modified into additional petals. There are many colours now available from pure milk white to pink, rose, and near red along with single to fully double forms. They are prolific bloomers, and have become the main source of peonies for the cut flower business (Halda *et al.*, 2004). In China, *P. lactiflora* is less highly valued as an ornamental plant than the cultivars of tree peony *Paeonia rockii* (tree peony) and *Paeonia suffruticosa*. In Mongolia, there are 116 tribes, 674 categories and 3014 species of plants, and of which 53 endangered species and 81 species of rare plants (Red book Mongolia, 2014). As well, there are more than 1,000 species of plants those are considered as an essential ingredient in medicine. In recent years, rare mineral and medicinal plants are threatened due to the uncontrolled use. *P.lactiflora* is a herbaceous perennial in the genus *Paeonia* family. The family Paeoniaceae is a perennial herb and widely distributed in Russia, Mongolia, Korea, Japan and China. This species occurs in Mongol Daguur, Khyangan (Grubov, 2014). A rhizome is an important Traditional Medicine and flowers of this plant are mainly used for ornamental purposes. In China, Korea, Japan and Mongolia, a decoction of the dries root without bark of *P.lactiflora* has been used in the treatment of

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rheumatoid arthritis, hepatitis, systemic lupus erythematosus, dysmenorrhea, muscle cramping, spasms and fever for more than 1200 years. A water/ethanol extract of the root is now known as total glucosides of *P.lactiflora, which* contains more than 15 bioactive components (Dong and Sheng 2011). *P.lactiflora* is included in the List of Very Rare Plants and Mongolian Red Book (1997, 2014) as Very Rare (Red book Mongolia 2014). There are two kinds of research works in *P.lactiflora*. The first one is about the influencing factors to number and diameters of flowers. Moreover, since it is an essentially important medicinal plant, the focus of the research works was on the biologically active compounds. Unfortunately, the research on its water relation and water potential of this plant are very rarely conducted. Therefore, we have decided to conduct the comparison study on of the water relation, chlorophyll fluorescence, and the simple phenolic content in the wild and the cultivated Mongolian very rare plant of *P.lactiflora*. Based on this kind of scientific research, the ideal usability of wild and cultivated plants of *P.lactiflora*, can be determined. It was the first comparison study on physiology and biochemistry of wild and cultivated plants *P.lactiflora* in Mongolia.

3 MATERIAL AND METHODLOGY

Morphology: Perennial herbs, 70-90 cm tall. Proximal leaves 2-ternate, terminal leaflets often 2 or 3 segmented, lanceolate or over lanceolate, 5-15 cm long, 1.5-5 cm wide, sparsely pubescent abaxially, margin white cartilaginous denticulate, apex acuminate. Flowers 20 cm diameters, white or pink (Figure 1).

Distribution: Khyangan, Dornod, Mongol Daguur.

Habitat: This species grows in meadow slopes, steppe and forb meadows, thinned shrubberies at slopes of hills, river valleys and foothills.

Parts Used: Root. The plant *P.lactiflora is* also used to treat dementia, headache, spasm of calf muscle, vertigo. Traditionally, *P.lactiflora* plant is used in treatment of atopic eczema boils and sores; to reduce fever, induce sterility and treat burns.

Constituents: The dried root of *P.lactiflora* contains 3.5-5.7% of paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin, albiflorin, pentagalloylglucose.

Figure 1: *Paeonia lactiflora* Pall.

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A. Determination of the water regime and water potential indices: The plant water status was assessed by conducting measurements of the shoot water potential on rainless days with a clear sky, using a Model 1515D Pressure Chamber Instrument (PMS Instrument Company, Albany, Oregon) and applying the method of Scholander et al. (Scholander, *et. al.* 1964). We studied the movement of stomata by using pattern method of Molotkovskii (Vicktorov, 1969). We used the fast weight-method by Ivanov, Silina, Tselinikher to define the transpiration intensity (Vicktorov, 1969). The water content and water deficit were defined by the method of D.Chatskii (1960) and the absolute water content and relative water content were defined by the method of Byambasuren et al (Byambasuren, 2010).

B. Determination of the chlorophyll fluorescence and chlorophyll index: The chlorophyll fluorescence indices were measured by Fluor Pen (FP 100). Optimal quantum yield (Schreiber and Bilger, 1993) is content of $CO_{2,\ \text{which}}$ is inspected to one solar quantum. If this value is, high that means this plant uses enough light energy. The maximum value of optimal quantum yield (QY) is 0.832 in C_3 plants. The chlorophyll fluorescence decrease ratio (Rfd) shows the photosynthetic activity. If Rfd≥2.5, it shows that photosynthetic activity is normal and if Rfd<1 it shows that photosynthetic activity is not enough (Mohammed, 1995, Rinderle and Lichtenthaler, 1988).

$$P = \frac{Ft}{1 - QY} \qquad Rfd = \frac{P - Ft}{Ft}$$

Chlorophyll index. The SPAD 502 Chlorophyll Meter instantly measures chlorophyll content (index) or "greenness" of our plants. The SPAD 502 Plus quantifies subtle changes or trends in plant health, long before they are visible to the human eye. Non-invasive measurement; simply clamps the meter over leafy tissue and receives an indexed chlorophyll content reading (-9.9 to 199.9) in less than 2 seconds.

C. Determination of simple phenolic content

Plant extracts were prepared according to a standard protocol. The simple phenolic content was determined by spectrophotometry using Gallic acid as a standard, accordingly to the method described by Singleton and Rossi (1965). The simple phenolic content was expressed as Gallic acid equivalents in mg/100ml of stem and leaves. The concentration of polyphenols in samples was derived from a standard curve of Gallic acid ranging from 100 to 500 μg/ml (Keskin-Šašić, 2012).

4 RESULTS AND DISSCUSSION

We have done the comparison study of simple phenolic content, indices of chlorophyll fluorescence (Ft, QY, Rfd), chlorophyll index, water potential and water regime indices in the wild (Valley of Tseent in Dadal soum of Khentii province, h=915m, 48°59.803'N, 111°46.817'E) and the cultivated (Botanical garden of Ulaanbaatar city) plants of *P.lactiflora* in July 2015.

A. Comparison water potential and water regime indices: We have determined the daily real water content, water deficit, absolute water content, relative water content and water potential in wild and cultivated plants of *P. lactiflora* during the day with 2 hours intervals. The expression of daily fluctuation was clearer in the wild plant than in the cultivated plant. Particularly, the water deficit and absolute water content were positively correlated (P=0.0001) with each other. The indices were increased from 6 AM and reached the maximum value at 18 PM. In addition, these indices were decreased from 20 PM and by 4AM they were at the minimum value. These indices were in an opposite correlation (P=0.0001) with the relative water content, actual water content and water potential. The relative water content, actual water content and water potential were in an opposite correlation with each other and these indices were decreased from 6 AM and reached the

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minimum value at 18 PM. Again, these were increased from 20 PM and were at the maximum value by 4 AM (Table 1, Figure 2). It was related that, water supply is relatively higher in early morning and during the night than during day. It could be the directly related to the environmental and climate factors. Namely during the day, the light intensity and air temperature are high and the relative air moisture is low but, without daylight or at night the relative air moisture is high and air temperature is low. By then, plants have possibility to absorb the losing water from soil solutions at nighttime.

Table 1: Comparison study of daily water regime indices in wild and cultivated plants of *P.lactiflora*

	Absolute water content		**Water deficit**		**Relative water content**		**Actual water**	
Hour	Wild	Cultivated	Wild	Cultivated	Wild	Cultivated	Wild	Cultivated
6	70.1±0.2	71.9±0.4	10.5±0.8	14.7±0.3	96.5±0.2	91.96±1.9	67.7±0.1	69.0±0.4
8	70.2±1.8	71.7±0.4	12.1±4.5	14.2±2.8	96.1±1.4	95.89±3.2	67.4±0.9	68.7±9.1
10	69.7±0.3	71.1±0.8	21.3±1.6	12.5±3.7	92.4±0.7	96.02±1.2	64.5±0.8	68.3±1.1
12	70.7±0.6	71.0±0.3	20.7±1.1	15.0±1.6	92.9±0.5	95.13±0.6	65.7±0.9	67.6±0.5
14	70.9±0.8	73.2±1.0	23.9±5.1	14.9±3.6	91.6±2.4	95.45±1.3	65.0±2.4	69.9±1.9
16	71.6±0.2	70.7±0.9	25.2±0.1	12.0±3.7	91.0±4.3	96.15±1.2	65.1±3.3	68.0±0.3
18	72.1±1.0	70.8±2.5	27.9±9.7	14.0±1.2	90.0±0.4	95.5±0.3	64.9±2.4	67.6±2.5
20	71.5±0.5	71.3±0.6	15.0±1.7	11.9±0.1	95.21±0.5	96.3±0.1	68.1±0.4	68.7±0.6
22	69.3±1.0	71.2±0.2	14.8±1.8	10.8±1.1	94.93±0.5	96.6±0.4	65.8±0.6	68.8±0.5
0	69.9±1.1	72.3±3.7	10.3±5.6	7.9±2.3	96.63±1.8	97.7±0.4	67.5±1.2	70.6±0.3
2	69.5±1.7	70.8±0.9	10.3±1.5	6.64±1.9	96.62±0.5	97.9±0.6	67.1±1.6	69.4±0.7
4	69.4±1.5	70.6±0.8	10.1±1.0	9.55±1.2	96.66±0.2	96.9±0.3	67.1±1.4	68.4±0.5
Average	70.5±0.9	71.5±0.8	17.4±6.6	12.2±2.8	93.9±2.5	95.9±1.5	66.3±1.3	68.7±0.9

We have compared the average indices of water regime between wild and cultivated plants of *P.lactiflora*. There were not clear differences between real water, absolute water content and relative water content but, water deficit was higher (P=0.01) and water potential was lower (P=0.0001) in wild plant than in cultivated plant (Figure 2, 3). In addition, we have determined the water potential when the turgor was equal to zero in wild and cultivated plants of *P.lactiflora*. The average water potential of the wild plant was lower (P=0.01) than the zero turgor was equal to zero. However, the average water potential of cultivated plant was higher (P=0.0001) than the zero turgor (Figure 4). These results showed that the cultivated plant has grown with irrigation and enough water condition, that is why the water deficit was low and water potential was high in the cultivated plant. The wild plant has grown in insufficient water condition so, the water deficit was high and water potential was low in the wild plant.

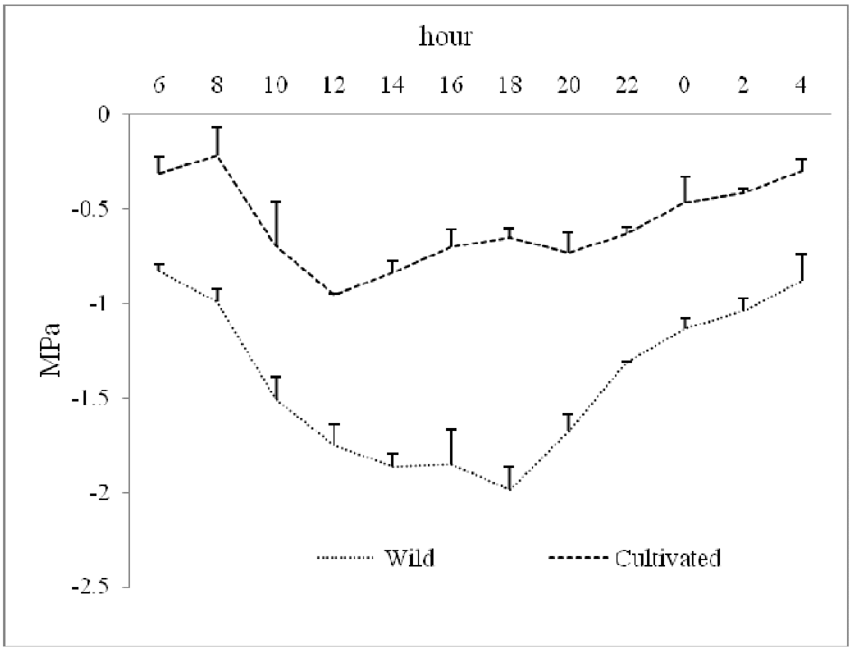

Figure 2: Daily water potential in wild and cultivated plants of *P.lactiflora*

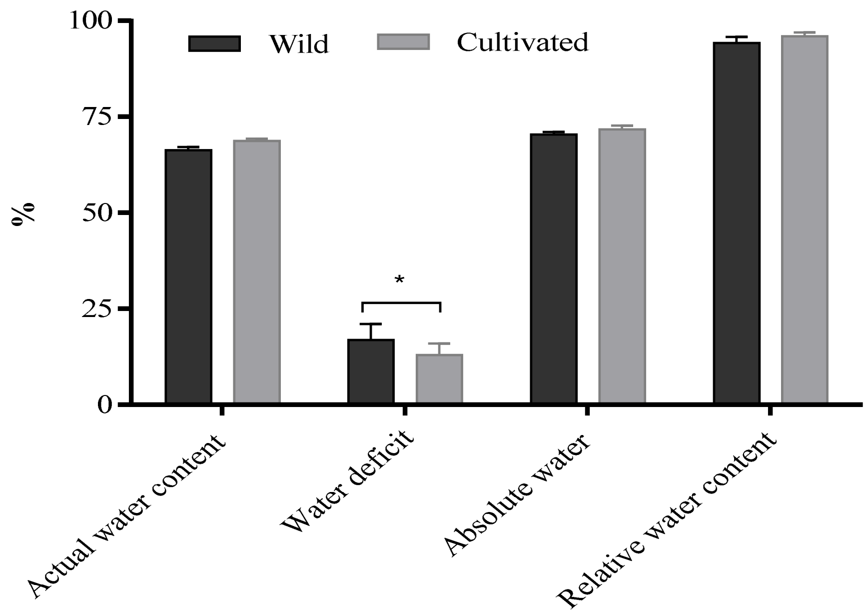

Figure 3: Comparison water regime indices in wild and cultivated plants of *P.lactiflora*

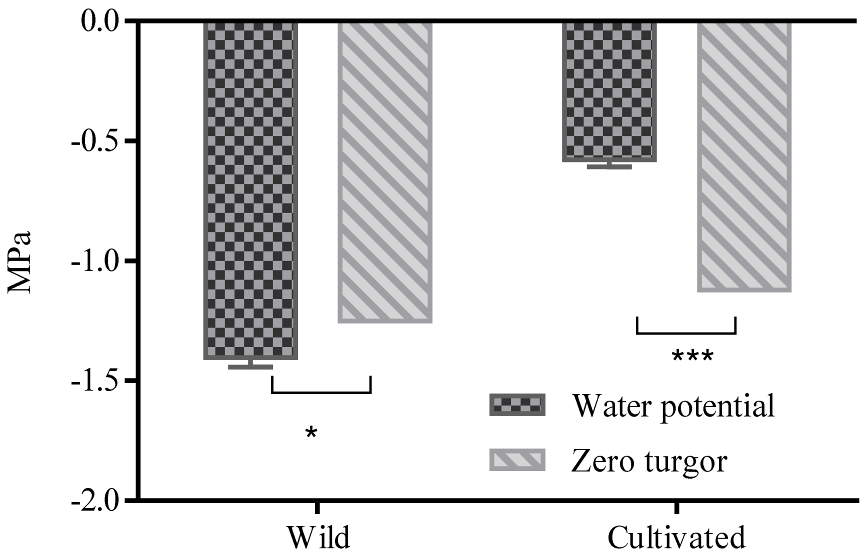

Figure 4: Comparison of water potential and zero turgor in wild and cultivated plants

B. Comparison of chlorophyll fluorescence indices and chlorophyll index: We have determined the daily chlorophyll fluorescence (Ft) optimal quantum yield (QY) and ratio fluorescence decrease (Rfd) of *P. lactiflora* with 2 hours intervals. Our research result has showed that the optimal quantum yield (QY) and ratio fluorescence decrease (Rfd) of *P. lactiflora* have a strong positive correlation (0.97-0.99) during the day and these indices were high in the morning and evening but were low in the afternoon (Figure 5). It was related to a concern that the rate of sunlight and air temperature were the highest at midday and afternoon and by then the plant stomata was closed therefore, it was not possible to absorb carbon dioxide for photosynthesis. Indices of optimal quantum yield (QY) and ratio fluorescence decrease (Rfd) were higher in the cultivated plant than in the wild plant ((Table 2) at the same time of morning (from 8AM to 12AM) and when the photosynthesis activity was high. This result showed that photosynthetic activity is relatively low in the wild plant. In addition, the chlorophyll index is higher in the cultivated plant (54.5±7.2) than in the wild plant (46.3±2.53).

Table 2. Chlorophyll fluorescence indices in cultivated and wild plants *(P.lactiflora)*

Plant	Light			Dark (20 minute)			Chlorophyll index
	Ft	QY	Rfd	Ft	QY	Rfd	
Wild	3462±161	0.66±0.02	2.07±0.06	3156±610	0.50±0.04	1.21±0.25	46.3±2.53
Cultivated	3514±735	0.70±0.02	2.31±0.24	5954±1987	0.70±0.02	2.59±0.32	54.5±7.2

This result showed that chlorophyll index and photosynthetic activity were higher in the cultivated plant than in the wild plant. It is related to that cultivated plant has grown in a condition with enough water.

C. Comparison of simple phenolic content: We have determined the content of simple phenolic and the content of stem, leaf and tuber were analyzed during seedling (July) the wild (Dadal sum, Khentii province) and the cultivated (in Botanical garden) *P.lactiflora*. (Figure 6). The

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simple phenolic content was lower in stem, leaf and tuber of the wild plant than in the cultivated plant. The physiological process of cultivated plant was found more active than the wild plant. In addition, the simple phenolic content was found higher in the cultivated than in the wild plant. It is related to that the cultivated plant has grown with the sufficient irrigation. In particular, as reported the phenolic compounds could be associated with anti-oxidative action in biological systems and acting as scavengers of singlet oxygen and free radicals (Jorgensen, 1999, Rice-Evans, 1995). The antioxidant potential of phenolic compounds can be attributed to their strong capability of transferring electrons to ROS/free radicals, chelating metal ions, active antioxidant enzymes and inhibitory oxidases (Cos, *et. al., 1998*).

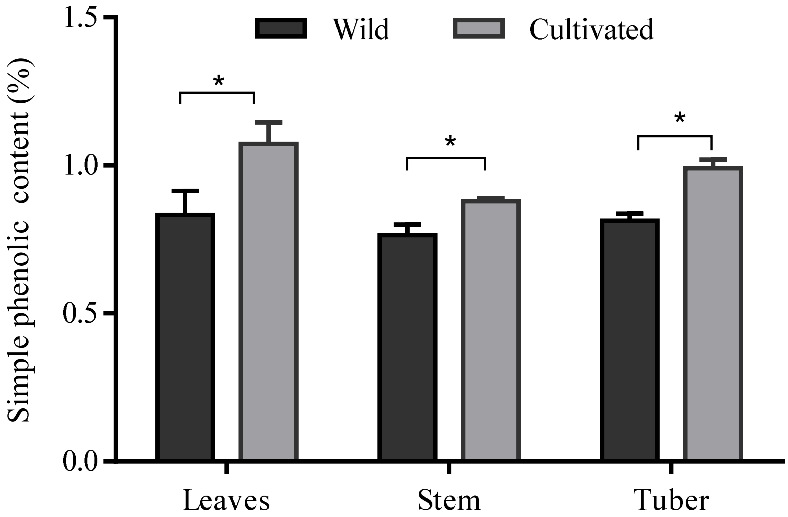

Figure 6: Simple phenolic content in the wild and cultivated *P.lactiflora*

5 CONCLUSION

The expression of a daily water regime indices fluctuation was clearer in the wild plant than in the cultivated plant. The water deficit and absolute water content were positively correlated with each other and these indices were in an opposite correlation with the relative water content, actual water content and water potential. It could be directly linked to the environmental and climate factors. There were not clear differences between the actual water, absolute water content and relative water content of wild and cultivated plants. Maybe, these indices are relatively stable in two different conditions for one species. The water deficit was lower and water potential was higher in the cultivated plant (-0.577± 0.23 MPa) than in the wild plant (-1.403±0.41 MPa). This result showed that the cultivated plant has grown in a condition with enough water. The optimal quantum yield (QY) and ratio fluorescence decrease (Rfd) of *P. lactiflora* were in a strong positive correlation (0.97-0.99) during day and these indices were high in the morning and evening, but were decreased in the afternoon in wild and cultivated plants. It has raised a concern that, the rate of sunlight and air temperature was the highest at midday and afternoon and by then plant stomata was closed therefore it was not possible to absorb carbon dioxide for photosynthesis. The optimal quantum yield (QY) and ratio fluorescence decrease (Rfd) were higher in the cultivated plant (0.70 and 2.59) than) in the wild plant (0.50 and 1.21 respectively. Also, the chlorophyll index is higher in the cultivated plant (54.5±7.2) than in the wild plant (46.3±2.53). Our result showed that physiological process of cultivated plant is more active than the wild plant and the simple phenolic content was about 1 time higher in the leaves, stem, tuber of cultivated than in the wild plant. In addition, this led to our conclusion of using the cultivated plant is possible. If the usage of cultivated plants then, the resource and raw medicinal materials will increase. Eventually, it could be the optimal solution for conserving the natural wild plants from becoming extinct.

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