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GROWTH AND PHYSIOLOGICAL RESPONSES OF MUGWORT (*Artemisia vulgaris* L.) TO DIFFERENT IRRIGATION FREQUENCIES

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ABTRACT

A pot experiment was conducted in a net house to evaluate the effects of three different irrigation frequencies (irrigation at a 7-day interval (T1), a 14-day interval (T2) and a 21-day interval (T3)) on the growth of three mugwort accessions. Results from the experiment revealed that different irrigation frequencies significantly affected the agronomical, anatomical, and physiological characteristics of mugwort. Decreasing the irrigation frequency from a 7-day interval (T1) to a 21-day (T3) led to significant decreases in plant height, leaf number, leaf area, and dry matter accumulation of each mugword accession. The highest value of plant dry weight was recorded in T1G1 (5.98g plant^{-1}) and the lowest was observed in T3G1 (2.53g plant^{-1}). Among the three mugwort accessions used in the study, G6 was seen to have the highest drought tolerance performance, followed by G1 and G7, respectively.

Keywords: *Artemisia vulgaris* L., irrigation frequency, mugwort.

Phản ứng của cây ngải cứu (*Artemisia vulgaris* L.) với các tần suất tưới khác nhau tại Gia Lâm, Hà Nội

TÓM TẮT

Thí nghiệm được tiến hành trong nhà lưới có mái che tại Học viện Nông nghiệp Việt Nam nhằm đánh phản ứng của cây ngải cứu với các khoảng cách tưới khác nhau thông qua một số chỉ tiêu nông học, sinh lý. Công thức xử lý hạn thông qua khoảng cách giữa các lần tưới hạn T1 (1 tuần/lần), T2 (2 tuần/lần), T3 (3 tuần/lần). Kết quả cho thấy tăng dần khoảng cách giữa các lần tưới đã làm giảm chiều cao cây, số nhánh, diện tích lá và khả năng tích lũy chất khô. Trong điều kiện thiếu nước kéo dài, bộ rễ cây ngải cứu có xu hướng tăng số lượng rễ cấp 1, tăng chiều dài và chiều rộng bộ rễ cũng như tăng hàm lượng diệp lục b. Trong điều kiện thiếu hụt nước đã làm giảm số lượng bó mạch rễ của cây ngải cứu. Khả năng chịu hạn của các mẫu giống tham gia thí nghiệm được xếp theo thứ tự G6 > G1 > G7.

Từ khóa: *Artemisia vulgaris* L., hạn, ngải cứu.

1. INTRODUCTION

The practice using of plants as a means of creating medicinal substances has been seen in many civilizations since ancient times, and plays a central role in folk medicine. It has been estimated that 80% of the world's population relies solely on traditional herbs as the source for primary health care (Annon, 2008). Among medicinal plants, mugwort (*Artemisia vulgaris* L.) appears to be one of the most commonly used and has proven itself to have a wide range of medicinal applications. In Chinese medicine, mugwort is used as a means for pain relief, and wound and bronchitis treating. More importantly, recent studies have revealed the effectiveness of using mugwort as tonics, antimalarials, antihelmintics, and antidiabetics (Uzun *et al.*, 2004).

Recently, most of the research carried out in the leaves of mugwort has had aims of evaluating its medicinal effects (Uzun *et al.*,

2004) and chemical composition (Umano *et al.*, 2000; Judžentienė and Buzelytė, 2006). Although there is an increasing demand for mugwort for medical materials as well as food, the production of mugwort has been found to be restricted by the cold and dry weather during winter in the north of Vietnam. To date, there exists a lack of understanding in how mugwort responds to water deficit and drought. This experiment aims to assess the growth and physiological responses of three mugwort accessions to different irrigation frequencies. The results from this experiment can provide a basis for developing strategies to maintain stable yields of mugwort in northern Vietnam.

2. MATERIALS AND METHODS

2.1. Plant materials

Three mugwort accessions collected in the north of Vietnam (G1, G6 and G7) were used in this experiment. Brief morphological descriptions of each accession are given in Table 1.

2.2. Experiment design

Three irrigation frequencies, at 7-day (T1), 14-day (T2), and 21-day (T3) intervals, and 3 mugwort accessions (G1, G6, G7) were employed in this study (Table 2). A 3 x 3 factorial experiment (9 treatments) was carried out following a split plot design with 3 replications (20 pots were counted as a replication). Mugwort seedlings (with 2 leaves and 10cm in height) were transferred into pots (18 x 20 x 25 cm) containing 3 kg of alluvial soil. Each pot was irrigated with 300ml of tap water.

2.3. Measurements

Leaf wilting (%) and recovery (%) of mugwort were measured at 3 and 7 days after re-irrigating.

At the 120th day of the experiment, 12 plants from each treatment were collected for the measurement of growth parameters, including: stem height (cm), no. of leaves plant^{-1}, no. of branches per plant^{-1}, stem diameter (cm), leaf area (dm^2 plant^{-1}), no. of adventitious roots, adventitious root diameter (cm), root length (cm), root width (cm), and dry weight accumulation (g plant^{-1}).

Chlorophyll a content (mg g^{-1}), chlorophyll b content (mg g^{-1}), and carotenoid content (mg g^{-1}) were measured followed Arnon (1949).

Anatomical parameters were observed and measured in plants on the 120th day of the experiment using the double staining method (Methylene Blue and Carmine). The no. of bundle sheathes in each root was recorded.

Table 1. Brief morphological descriptions of 3 mugwort accessions

Accession	Color			Hairiness			Origin
	Stem	Leaf blade	Leaf Vein	Stem	Upper leaf surface	Lower leaf surface	
G1	Purple	Green	Purple	None	None	None	Thuan Chau- Son La
G6	Purplish green	Green	Green	Very dense	Very dense	Very dense	Thai Thuy-Thai Binh
G7	Purplish green	Green	Green	Very dense	Slight	Very dense	Thuan Chau-Son La

Table 2. Nine treatments used in the study

Accession	Irrigation frequency		
	T1 (irrigation at 7-day interval)	T2 (irrigation at 14-day interval)	T3 (irrigation at 21-day interval)
G1	T1G1	T2G1	T3G1
G6	T1G6	T2G6	T3G6
G7	T1G7	T2G7	T3G7

2.4. Data analysis

All data collected in the study were subjected to Analyses of Variance (ANOVA) using Microsoft Excel and Cropstat (version 7.2).

3. RESULTS AND DISCUSSIONS

3.1. Effects of irrigation frequency on leaf-wilting and recovery of mugwort

One of the early responses to drought developed by plants is the dehydration of water in the leaves. Besides, a water deficit in the leaf surface leads to the loss of water in guard cells together with a decline of turgidity and thus, makes stomata pores close (Hightshoe, 1987). Dehydration and loss of water in leaves finally results in leaf wilting. Levels of leaf wilting vary and can be grouped into: incipient (i), temporary (ii), and permanent (iii) (Hightshoe, 1987). While incipient wilting doesn't lead to leaf drooping, temporary wilting causes leaf drooping during the day and recovery during night time. Prolonged and sustained drought can make leaves permanently wilted and unable to recover during night time. Leaf recovery from permanent wilting therefore, needs thorough re-irrigation of the soil (Hightshoe, 1987). In addition, permanent leaf wilting is believed to have many adverse effects on different aspects of plant growth including photosynthesis, respiration, and leaf transpiration (Athar and Ashraf, 2005). Early drought responses can be seen by observation of leaf wilting and recovery (recorded at 3 and 7 days after re-irrigation).

Data regarding mugwort leaf wilting and recovery are presented in Table 3. Among the three irrigation frequencies, plants grown under T3 (irrigation at a 21-day interval) obtained a higher rate of leaf wilting and lower recovery rate compared to plants grown under T1 and T2. Under T3, the highest value of leaf wilting was recorded in G1 (100.00%) followed by G7 (60.00%) and G6 (46.67%), respectively. However, compared to other plants, mugwort is believed to be relatively good at recovering from wilting. This can be explained by the fact that mugwort has white wooly hairs coating the leaf surface which helps to reflect heat and hence, reduce the loss of water in the leaves (El-Sahhar, 2010). This allows the leaves of mugwort to be able to withstand different levels of drought and maintain the plant's recovery. According to Roy *et al.*, (1999), leaf hairs play an important role in ameliorating the effects of imposed drought. An increase in leaf hair density reduced water loss from the leaf surface while a decrease in leaf hair density was believed to increase light reception (Roy *et al.*, 1999). Among the three mugwort accessions in this experiment, G6 showed highest drought tolerance potential. At 7 days after re-irrigation, under T3, G6 obtained the highest rate of recovery from wilting (80.00%), followed by G1 and G7 (66.67%). This may be mainly due to the dense layer of hairs on both the upper and lower leaf surfaces in G6. Compared to G1 and G7, G6 plants have the highest leaf hair density. Having dense hairs coated on both sides of the leaf allows G6 to highly reduce water loss from the leaf surface during prolonged water stress and hence, helps the plant to recover from wilting. This can be seen as one of the mechanisms employed by plants to better adapt to sustained water deficit and drought.

3.2. Effects of different irrigation frequencies on stem and leaf development of mugwort

Plants have developed various mechanisms to cope with water stress both at the cellular and whole-organism level (Farooq *et al.*, 2011). The effects of drought and water stress on plants are varied and can be detected by both morphological and molecular changes. It has been reported that drought and water stress led to reduced leaf size, decreased stem growth and root expansion, increased hair density on leaves and stems, and altered plant and water relations (Farooq *et al.*, 2011). Morphological and growth responses of mugwort to different irrigation frequencies are presented in Table 4.

Table 3. Effects of different irrigation frequencies on leaf wilting and recovery

Irrigation frequency	Accession	Leaf wilting (%)	Recovery (%)	
			3 days after re-irrigation	7 days after re-irrigation
T1	G1	13.33	100.00	100.00
	G6	6.67	100.00	100.00
	G7	6.67	100.00	100.00
T2	G1	33.33	80.00	100.00
	G6	13.33	100.00	100.00
	G7	20.00	66.67	100.00
T3	G1	100.00	53.33	66.67
	G6	46.67	60.00	80.00
	G7	60.00	55.56	66.67

Table 4. Effects of different irrigation frequencies on stem and leaf development of mugwort

Irrigation frequency	Accession	Main stem height (cm)	No. of branch plant^{-1}	No. of leaves plant^{-1}	Leaf area (dm^2 plant^{-1})
T1	G1	26.47	6.68	31.55	12.27
	G6	35.13	6.01	28.84	10.65
	G7	24.69	7.33	24.22	11.44
T2	G1	28.20	5.69	22.89	8.60
	G6	28.40	5.74	24.27	4.65
	G7	21.63	6.26	17.71	7.31
T3	G1	21.58	4.30	13.20	6.89
	G6	24.41	5.13	21.02	4.20
	G7	17.78	4.72	17.20	4.15
$LSD_{0.05\ G*T}$		1.70	1.15	3.32	0.93
Mean T	T1	28.76	6.67	28.20	`11.45
	T2	26.08	5.89	21.26	6.85
	T3	21.26	4.72	17.14	5.08
$LSD_{0.05\ T}$		1.57	0.46	1.47	0.61
Mean G	G1	25.42	5.56	22.55	9.25
	G6	29.31	5.63	24.71	6.50
	G7	21.37	6.10	19.71	7.63
$LSD_{0.05\ G}$		0.98	0.67	1.92	0.54
$CV\%_{\ G*T}$		3.8	11.3	8.4	6.7

Stem height is considered a variable trait and its expression is strongly influenced by environmental and technical factors (Zecevic *et al.*, 2008). According to Ninh Thi Phip *et al.*, (2015), in well-watered conditions, G6 has longer stem internodes compared to G1 and G7 and hence, often obtains greater values for stem height. In this study, different irrigation frequencies significantly affected stem growth and leaf development of mugwort. A lower frequency irrigation (T3) significantly decreased the stem height, number of branches plant^{-1}, number of leaves plant^{-1}, and leaf area of mugwort. The main value of stem height of mugwort was reduced from 28.76 (cm) in plants grown under T1, to 26.08 (cm) in T2, and was lowest at T3 (21.26 cm). Among the three accessions, the highest stem height was observed in G6 (29.31 cm), followed by G1 (25.42 cm) and G7 (21.37 cm), respectively.

Morphological adaptations of plants to drought and water stress have been reported in previous studies (Wu *et al.*, 2008; Kadiođlu *et al.*, 2012). Leaf adaptation is considered one of the most important factors favoring the success of a plant under drought and poorly-watered conditions. Statistical analysis (p = 0.05) revealed significant differences among mugwort accessions and irrigation frequencies in this study. Among the three irrigation frequencies, the lowest leaf number was observed in plants grown under T3 (17.14 leaves $plant^{-1}$), followed by T2 (21.26 leaves $plant^{-1}$) and T1 (28.20 leaves $plant^{-1}$), respectively. The largest reduction in leaf area was also recorded in plants grown under T3 (5.08 dm^2 $plant^{-1}$), followed by T2 (6.85 dm^2 $plant^{-1}$) and T1 (11.45 dm^2 $plant^{-1}$). Water stress caused significant reductions in leaf number and leaf area (LA) of mugwort, confirming the same results as previous studies in almonds (Zamani *et al.*, 2002; Khosroshahi *et al.*, 2014), peaches (Rieger *et al.*, 2003), and apples (Liu *et al.*, 2012). The reduction of leaf area can be seen as an important stress avoidance strategy and is considered the first defensive mechanism employed by plants to withstand different levels of drought (Khosroshahi *et al.*, 2014). Besides, depending on drought duration and intensity, a plant might minimize transpirational water loss by reducing its leaf number. Because individual leaf size was not affected by drought and water stress, the reduction of leaf area (LA) in mugwort is mainly caused by leaf abscission and reduced leaf number (Khosroshahi *et al.*, 2014).

3.3. Effects of different irrigation frequencies on root development of mugwort

The mugwort root system is characterized by light brown rhizomes (up to 1 cm in diameter), branching at nodes, mostly distributed in the upper 20 cm of the top soil. The rhizomes, together with adventitious roots developed from the nodes of each rhizome, form a complex and extensive underground structure (El-Sahhar, 2010). Root growth and development is strongly affected by drought and water stress. In addition, an extensive root structure is believed to be advantageous for plant growth under drought (Anjum *et al.*, 2011). Besides, root development is believed to enhance water uptake and together with higher proline content, helps the plants maintain a suitable osmotic pressure for survival and growth under drought stress (Djibil *et al.*, 2005). Reponses of mugwort to different irrigation treatments with regard to root growth and development in this study are presented in Table 5.

Data from Table 5 revealed that different irrigation frequencies significantly affected the root characteristics of mugwort (no. of adventitious root $plant^{-1}$, adventitious root diameter, root length, and root width). Among the three irrigation frequencies, the highest number of adventitious roots was found in plants grown under T3 (57.58 $plant^{-1}$), followed by T2 (49.92 $plant^{-1}$) and T1 (46.50 $plant^{-1}$), respectively. In addition, the highest values for root length and root width were also recorded in plants grown under T3. However, within the same irrigation frequency, no significant differences (p = 0.05) in root characteristics (no. of adventitious root $plant^{-1}$, adventitious root diameter, root length, and root width) were found among the three mugwort accessions. Water stress triggered the development of the mugwort root system by increasing the root length, increasing the number of adventitious roots, and reducing their diameter. Having a smaller diameter allows the adventitious roots to deeply penetrate into smaller soil pores (Franco, 2011) and thus, optimizes water uptake by the root system. This may be considered a key role for mugwort survival and growth under water stress.

Table 5. Effects of irrigation frequencies on root development of mugwort

Irrigation frequency	Accession	No. of adventitious root plant^{-1}	Adventitious root diameter (cm)	Root length (cm)	Root width (cm)
T1	G1	43.50	0.17	8.10	8.58
	G6	46.75	0.22	7.45	7.85
	G7	49.25	0.19	11.89	9.19
T2	G1	44.50	0.16	13.45	13.00
	G6	51.75	0.16	10.90	12.45
	G7	53.50	0.17	9.18	10.23
T3	G1	54.25	0.14	14.15	13.35
	G6	58.50	0.15	13.97	14.15
	G7	60.00	0.16	14.76	13.88
$LSD_{0.05\ G*T}$		4.32	0.06	1.07	1.42
Mean T	T1	46.50	0.19	9.15	8.54
	T2	49.92	0.16	11.18	11.89
	T3	57.58	0.15	14.29	13.79
$LSD_{0.05\ T}$		2.30	0.03	1.46	0.50
Mean G	G1	47.42	0.15	11.90	11.64
	G6	52.33	0.18	10.77	11.48
	G7	54.25	0.17	11.94	11.10
$LSD_{0.05\ G}$		2.50	0.03	0.62	0.82
$CV\%_{\ T*G}$		4.7	1.6	5.2	7.0

Compared to shoot growth, root growth is less influenced by drought and a decrease in the shoot:root ratio can be seen as an early response of plants under drought conditions (Franco, 2011). While shoot growth is rapidly reduced when plants are grown under low water potentials, roots maintain the ability to elongate when being subjected to low water potentials which totally inhibit the growth and development of the shoot (Wu and Cosgrove, 2000). In fact, root architecture (root structure and root distribution) contributes a larger part in determining drought tolerance than root quantity (Farooq *et al*., 2011). Breeding for a deep and extensive root structure of mugwort therefore, is highly recommended to reduce the effects of drought on its growth and yield.

3.4. Effects of different irrigation frequencies on photosynthetic pigments and dry weight accumulation of mugwort

Drought has been reported to cause a reduction in the content of photosynthetic pigments such as chlorophyll (chlorophyll *a* and chlorophyll *b*) and carotenoids (Mafakheri *et al*., 2011; Ashraf and Harris, 2013). Besides, severe drought leads to deterioration of thylakoid membranes (Anjum *et al*., 2011). Changes in photosynthetic pigments have important implications for drought tolerance in plants (Jallel *et al*., 2011).

While chlorophyll *a* (Chl *a*) content slightly decreased from 0.31 mg/g (T1) to 0.30 mg/g (T2 and T3), a considerable decline of chlorophyll *b* (Chl *b*) was observed across different irrigation frequencies. Chl *b* content declined from 0.74 mg/g (in T1) to 0.73 mg/g (in T2) and 0.64 mg/g (in T3). The reduction of photosynthetic content in leaves under drought has also been reported in wheat (Ashraf *et al*., 1994), canola (Din *et al*., 2011), cotton (Massacci *et al*., 2008), and sunflower (Kiani *et al*., 2008). The decline of chlorophyll content under drought and a water deficit is mainly due to the damaged chloroplasts caused by the accumulation of active oxygen species (ROS) such as hydroxyl radicals (OH), hydrogen peroxide

(H_2O_2), alkoxy radicals (RO), and anion radicals (O^{-2}) (Farooq *et al.*, 2009). ROS is believed to react with lipids and proteins, leading to destructive damage such as lipid peroxidation and chlorophyll bleaching (Terzi *et al.*, 2006). Since Chl *b* is more sensitive to drought than Chl *a*, a greater decline in Chl *b* is commonly observed in plants (Farooq *et al.*, 2009), and thus, leads to a rise in the Chl *a/b* ratio.

It's well reported that plants have developed both enzymatic and non-enzymatic defense mechanisms to alleviate and reduce damage caused by ROS. This is termed as antioxidant defense and can be seen as one of the important criteria for the screening of drought tolerant genotypes (Faize *et al.*, 2011). Among photosynthetic pigments, carotenoids have an additional role in protecting leaves from oxidative damage caused by ROS. Carotenoids are known to carry the function of photoprotectants by quenching ROS or dissipating heat of excess light energy (McElroy and Kopsell, 2009). Having a high content of carotenoids help the plants to better withstand the adverse effects caused by drought and water deficit. This is entirely consistent with the mechanism of mugwort to tolerate drought when the carotenoid contents recorded in plants with T2 or T3 (0.32 and 0.30 mg/g, respectively) were higher than that found in T1 (0.28 mg/g). A decline in carotenoid content in plants from T2 to T3 suggested that T2 is the threshold for drought tolerance of mugwort. Less frequent irrigation (than T2) or more severe drought can lead to disorders in metabolic processes and then further restrict the growth of mugwort.

Table 6. Effects of different irrigation frequencies on photosynthetic pigments and dry weight accumulation

Irrigation frequency	Accession	Pigment content (mg/g)			Dry weight (g plant^{-1})
		Chlorophyll *a*	Chlorophyll *b*	Carotenoid	
T1	G1	0.31	0.76	0.28	5.98
	G6	0.31	0.74	0.27	5.21
	G7	0.31	0.72	0.27	5.23
T2	G1	0.30	0.60	0.26	4.18
	G6	0.28	0.95	0.29	5.79
	G7	0.31	0.65	0.41	3.57
T3	G1	0.30	0.65	0.34	2.53
	G6	0.29	0.58	0.29	3.22
	G7	0.30	0.70	0.27	2.92
$LSD_{0.05\ T*G}$		-	-	-	0.34
Mean T	T1	0.31	0.74	0.27	5.47
	T2	0.30	0.73	0.32	4.51
	T3	0.30	0.64	0.30	2.89
$LSD_{0.05\ T}$		-	-	-	0.67
Mean G	G1	0.30	0.67	0.28	4.23
	G6	0.29	0.76	0.26	4.74
	G7	0.31	0.69	0.31	3.91
$LSD_{0.05\ G}$		-	-	-	0.20
$CV\%_{\ T*G}$		-	-	-	4.4

Table 7. Effects of different irrigation frequencies on root anatomy

Irrigation frequency	Accession	Number of bundle sheath		Total number (bundle root^{-1})
		Large bundle (bundle root^{-1})	Small bundle (bundle root^{-1})	
T1	G1	45	43	88
	G6	25	38	63
	G7	50	30	80
T2	G1	41	35	76
	G6	25	30	55
	G7	36	42	78
T3	G1	30	28	58
	G6	20	20	40
	G7	33	18	51
Mean T	T1	40.00	37.00	77.00
	T2	34.00	35.67	69.67
	T3	27.70	22.00	49.67
Mean G	G1	38.67	35.33	74.00
	G6	23.33	29.33	52.67
	G7	39.67	30.00	69.67

Data collected in the experiment also revealed the effects of drought on dry weight accumulation of mugwort. Drought significantly reduced the dry weight of mugwort from 5.47g plant^{-1} in T1 to 4.51 g plant^{-1} in T2 and 2.89 g plant^{-1} in T3. Within the same T3 treatment, G6 appeared to have better tolerance performance with the highest plant dry weight (4.74 g plant^{-1}), followed by G1 and G7 with 4.23 and 3.91 g plant^{-1}, respectively.

3.5. Effects of different irrigation frequencies on root anatomy

Water stress is well known to markedly affect anatomical features in different plant species. It significantly decreased leaf thickness, leaf hair number, xylem vessel area, and vascular bundles (Aldesuquy, 2013). Besides the structural alterations caused in xylem and phloem areas, water stress also leads to reduced xylem conductivity. In addition to the anatomical alterations in leaves, changes in root anatomy provide a deeper understanding of drought tolerance mechanisms in plants.

Data from this experiment revealed that a decrease in irrigation frequency led to decrease in the number of large and small vascular bundles and also in the total number of vascular bundles in the roots of the three mugwort accessions. Mean number of vascular bundles decreased from 77.00 bundles (in T1) to 69.67 bundles (in T2) and was lowest at 49.67 bundles (in T3). The number of vascular bundles varied across irrigation frequencies and mugwort accessions. Among the three accessions in this study, G1 appeared to have the highest number of vascular bundles under water stress (74.00 bundles root^{-1}), followed by G7 (69.67 bundles root^{-1}) and G6 (52.67 bundles root^{-1}).

4. CONCLUSIONS

Water stress significantly affected the agronomical, anatomical, and physiological characteristics of mugwort. A decrease in irrigation frequency from a 7-day interval (T1) to a 21-day (T3) led to significant decreases in plant height, leaf number, leaf area, and dry matter accumulation of each mugwort accession. The highest value of plant dry weight was recorded in T1G1 (5.98 g plant^{-1}) and the lowest was observed in T3G1 (2.53 g plant^{-1}). Among the three mugwort accessions in the study, G6 was seen to have the highest drought tolerance performance, followed by G1 and G7, respectively. Mugwort adapts to water stress by increasing the number of adventitious root, having a prolific root system, and maintaining its chlorophyll *a* content while increasing its chlorophyll *b* content.

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