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Original Article

Selection of vetiver grass based on growth and nutrient content under saline water irrigation and waterlogging prior to mutagenesis

Katarut Chusreeaeom* and Nualchavee Roongtanakiat

Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand

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Abstract

The effects of saline water irrigation and waterlogging on the growth and nutrient contents of 19 vetiver ecotypes were investigated. Plant height, tiller number, and dry biomass were recorded at 12 months after planting. The results indicated that plant growth and development were influenced by saline water irrigation and waterlogging conditions and were different among the vetiver ecotypes. The accumulation of sodium in the shoots was higher than in the roots under salinity conditions that would be of benefit as vetiver can be used to remove salt contamination from soil or water. Under waterlogging conditions, total phosphorus had a higher concentration in the roots due to the effect of root oxygen stress on phosphorus uptake. We screened 8 vetiver ecotypes that showed salt and flood tolerance. These ecotypes would be of benefit for crop improvement with the best available plant material in our breeding program.

Keywords: vetiver, plant growth, plant nutrient, abiotic stress, screening

1. Introduction

Vetiver grass is a graminaceous plant native to tropical and subtropical areas. It has good ability to adapt to extreme environments, for instance drought, submergence, acidity, alkalinity, salinity, and heavy metals (Dalton, Smith, & Truong, 1996; Ghotbizadeh & Sepaskhah, 2015; Roong tanakiat, Osotsapar, & Yindiram, 2009). Truong and Baker (1998) reported on vetiver tolerance to soil water stress and water salinity. As a result of global warming, Thailand has suffered from flooding many times in recent years. Flooding is a natural disturbance affected by heavy rainfall and river flow and it has serious implications for soil conservation and slope stabilization. Vetiver is an effective grass which is tolerant to waterlogging conditions and can prevent soil erosion (Inthapan & Boonchee, 2000; Truong & Baker, 1998). Not only flooding but soil salinity from the overflow of sea water to agricultural land is a common problem. Salinity is one of the environmental stresses on crop plants. Salinity from the soil and irrigation water is a problem that restricts yield in arid and coastal areas. Cuong, Minh, and Truong (2015) reported that vetiver could grow in saline water (0-19.64 dS/m) and it is quite suitable for treating polluted water and is resistant to water or soil salinity.

In Thailand His Majesty the Late King Bhumibol Adulyadej, long realized the problem of soil degradation. He actively promoted the use of vetiver to preserve soil and water because it has a long fibrous root system that can penetrate deep into the soil. Roongtanakiat *et al.* (2009) reported that two species of vetiver, *Chrysopogon nemoralis* (Balansa) Holttum and *Chrysopogon zizanioides* (L.) Roberty have distinct ecological characteristics which help them adapt to different habitats. In particular, *Chrysopogon nemoralis* (Balansa) Holttum is found only in Southeast Asia, (Thailand, Laos, Cambodia, Vietnam, and Malaysia). It has a limited area of distribution and is usually found in dry areas or in soil with good drainage conditions in all regions of Thailand except in southern Thailand. It can adapt in areas with both strong or moderate sunlight and the tip of the shoot bows like lemon grass. *Chrysopogon zizanioides* (L.) Roberty can rapidly adapt to adverse environments as can most vetiver ecotypes, including those imported from India, Sri Lanka, and Indonesia (Office of the Royal Development Projects Board [ORDPB], 1998). The objectives of this study were to determine the

*Corresponding author
Email address: fscikac@ku.ac.th

impact on 19 vetiver ecotypes of saline water irrigation and waterlogging on their growth and dry biomass as well as to analyze the concentrations of plant nutrients including total nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) in the shoots and roots.

2. Materials and Methods

2.1 Pots experiment

Vetiver ecotypes have been named after the province or country where they were first found. In this study, 19 vetiver ecotypes were grown under saline water irrigation and under continuous submergence: Maehae; Maihuywai; Japanese; Monto; Prachuap Khiri Khan; Fiji; Ratchabura; Kamphaeng Phet 1; Kamphaeng Phet 2; Sri Lanka; Prarat Chathan; Roi Et; Loei; Nakhon Sawan; Syuntangbaimai; Nangpaya; Surat Thani; Songkhla 3; and Dyunrimtang. The experiment was carried out at the Nuclear Technology Research Center, Faculty of Science, Kasetsart University, Bangkok, Thailand using a completely randomized design (CRD) with three replications. Under saline water irrigation, the vetiver tillers were planted in pots containing 2 kg of sandy soil. The 0–15 cm layer of soil had pH 6.6, 0.03% organic matter, 64.18 µS/cm electrical conductivity and 41.03, 20.23, 356.05, and 38.6 mg kg^{-1} of available-P, extractable-K, extractable-Calcium, and extractable magnesium (Mg), respectively. Two weeks after planting, the plants were irrigated with 50 mL of sea water. The average chemical composition of the sea water used in these experiments is shown in Table 1. Under waterlogging conditions, the vetiver tillers were planted in pots containing 2 kg of Hupkaphong series sandy soil (coarse-loamy, siliceous isohyperthermic Ustoxic Dystripepts). The 0–15 cm layer of soil had pH 5.5 (1:1, H_2O:soil), 0.8% organic matter (Walklay & Black, 1934) and 0.77, 11.00, and 68.00 mg kg^{-1} of available-N, available-P, and extractable-K, respectively. Two weeks after planting, the plants were submerged in tap water at a maximum water level of 10 cm above the soil surface. The control was performed under normal conditions using pots containing 2 kg of Hupkaphong series sandy soil. Weeds were controlled using hoeing as necessary. The plant height and the tiller number were recorded at 12 months after planting under salinity and waterlogging conditions. After harvesting, each plant was divided into shoots (upper ground part) and roots (lower ground parts). The dry biomass of each plant sample was determined after oven drying at 80 ºC to a constant weight.

2.2 Primary nutrition analysis

The total N, P, K, and Na concentrations were determined independently for each replication compared to the control, shoot, and root. The primary nutrient analysis was conducted in the laboratory of the Division of Soil Science, Department of Agriculture, Bangkok, Thailand. Total N was determined using distillation and titration of NH_3 in solution (Bradstreet, 1965). Total P was determined using colorimetry (blue molybdophosphoric acid method; Olsen & Dean, 1965) and total K and Na were measured using atomic emission spectrometry (Lierop, 1976; Thomas, Sheard, & Moyer, 1967).

2.3 Statistical analysis

The data were subjected to analysis of variance (ANOVA) according to the CRD with three replications for each treatment and 19 samples for each replication. Where the main effects were significant, mean separation was carried out using the least significant difference (LSD) test.

3. Results and Discussion

3.1 Plant growth

The vetiver samples grown under non-stress conditions (control) had similar growth levels and almost all grew well. The Japanese ecotype was the tallest and the Nakorn Sawan, Prachuap Khiri Khan, and Songkhla 3 were the shortest (Figure 1a). Vetiver samples grown under the stress conditions of saline water irrigation and waterlogging were capable of growth and producing new tillers; however, there was a decrease in the height and tiller number compared to the control and the decreases differed among the vetiver ecotypes. Under salinity conditions, the Nakhon Sawan ecotype was the tallest followed by Sonkhla 3 and Sri Lanka, whereas the Dyunrimtang ecotype was the shortest (Figure 1b). Under waterlogging conditions, the growth level of all 19 ecotypes varied greatly in growth level compared to under normal and salinity conditions. The Rachaburi ecotype was the tallest followed by the Japanese and Songkhla 3 ecotypes, whereas the Surat Thani ecotype was the shortest (Figure 1c).

The 19 vetiver ecotypes had high tillering ability when grown under the non-stress conditions, producing between 4 and 9 tillers per pot. The Surat Thani, Kamphaeng Phet 2, and Nakhon Sawan ecotypes demonstrated high tillering ability under non-stress conditions but the Ratchaburi produced the lowest tiller number (Figure 2a). Under saline water irrigation, vetiver samples could still grow and develop, though their tillering ability was reduced. All 19 vetiver ecotypes produced fewer than four new tillers per pot. Monto and Sri Lanka had high tillering under saline water irrigation. Nonetheless, there were no significant differences among the ecotypes excluding Prachuap Khiri Khan, Ratchaburi, Syuntangbaimai, and Nangpaya (Figure 2b).

Table 1. Average chemical composition of sea water used in the experiment.

Constituent	pH	meq/L								SAR[1/]
		Ca	Mg	Na	K^+	Cl^-	CO_3^{2-}	HCO_3^-	SO_4^{2-}	
Sea water	7.9	19.05	100.49	441.30	9.60	518.88	0.80	1.6	50.67	57.08

[1/]Sodium absorption ratio

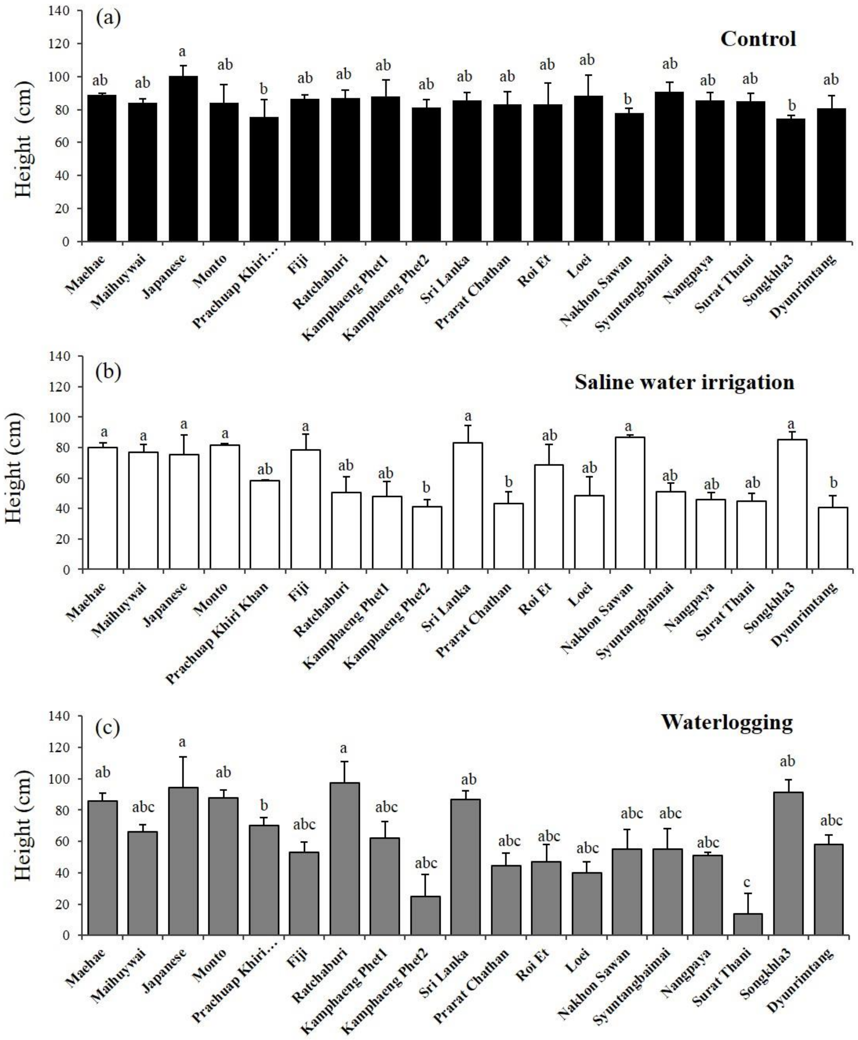

Figure 1. Average height of 19 vetiver ecotypes grown under (a) non-stress condition (control), (b) saline water irrigation and (c) waterlogging conditions. (error bar shows ±SD and columns with a common lowercase letter are not significantly different at the 0.05 probability level using LSD).

Accordingly, salinity conditions affected the tillering ability of vetivers. Under waterlogging conditions, the tillering ability varied greatly among vetiver ecotypes. The Maehae, Monto, Japanese, and Sri Lanka ecotypes showed high tillering ability under the waterlogging condition but Kamphaeng Phet 2 and Surat Thani were severely affected by submergence (Figure 2c). Remarkably, the Maehae, Japanese, Monto, Ratchaburi, and Sri Lanka ecotypes showed good adaptation to submergence. Similarly, Chinapan, Sukhasem, and Moncharoen (1992) studied 27 local vetiver ecotypes and found that the Sri Lanka ecotype produced high tiller numbers and good characteristics for clay loam and laterite soil. Cuong *et al.* (2015) and Ghotbizadeh and Sepaskhah (2015) reported that growth of vetiver in terms of shoot height and number of new shoots decreased as the salinity increased.

3.2 Dry biomass

Growth and high biomass are key factors in selecting effective ecotypes for growth under stress conditions. The dry weights of the shoots and roots under saline water irrigation and waterlogging conditions were also examined. The dry biomass of vetiver under waterlogging conditions was much higher and had greater variation than under salinity conditions which ranged from 27.58 to 2.09 g in the shoots and from 41.08 to 1.82 g in the roots. Sri Lanka had much greater shoot and root biomasses than the other ecotypes under submergence. Ratchaburi also had high biomass, followed by the Monto, Maehae, Songkhla 3, Japanese, and Khamphaeng Phet 2 ecotypes. In saline water, the Fiji and Japanese ecotypes had the highest dry biomass in the shoots and roots, respectively (Table 2). The biomass of vetiver roots is relevant to soil stabilization; however, in terms of usage and application the main focus with vetiver biomass is generally on the shoots. Vetiver is highly efficient at converting solar radiation into biomass since it is a C4 plant. The biomass of the vetiver shoot has multifunctional use, for example as animal feed, mushroom culture, roof thatching, and composting (ORDPB, 1998). Nevertheless, there are several factors affecting the biomass, for example low concentration

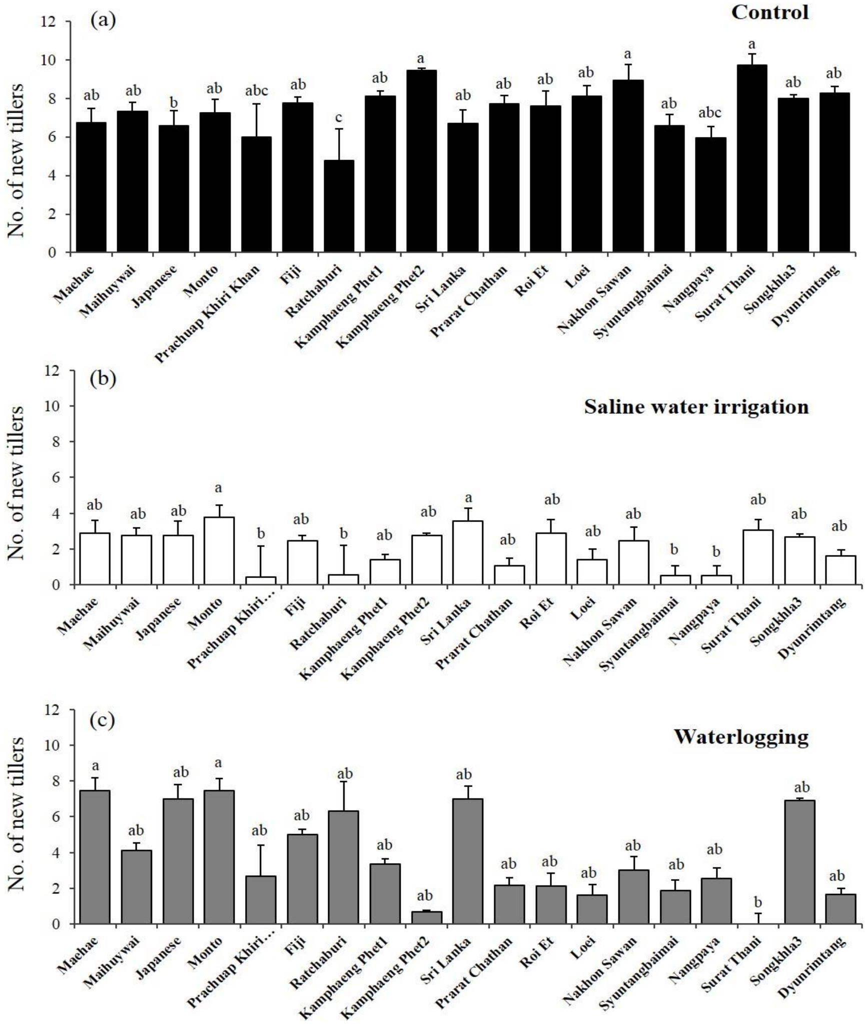

Figure 2. Average tiller number of 19 vetiver ecotypes grown under (a) non-stress condition (control), (b) saline water irrigation and (c) waterlogging conditions. (error bar shows ±SD and columns with a common lowercase letter are not significantly different at the 0.05 probability level using LSD).

of soil nutrients, acidic or saline soil, shading, and submergence (Menahem, Zvi, Zativ, & Meni 2009; Mohammad, Sydonia, Timothy, Dana, & Clancy, 2018).

The Department of Land Development reported that 10 of 28 ecotypes have proven suitable to grow in various soil types (ORDPB, 2000). As a result, *C. nemoralis* species (Nakorn Sawan, Kamphaeng Phet 1, and Ratchaburi) were identified as suitable to grow in clay loam soil and sandy soil whereas Prachuap Khiri Khan, Loei, and Monto were suitable for lateritic soil. Moreover, the *C. zizanioides* species (Kamphaeng Phet 2) is suitable to grow in lateritic soil and sandy soil. Sri Lanka is suited to lateritic soil whereas Songkhla 3 is suitable for all soil types. Our results verified that plant height, tiller number, and dry biomass under saline water irrigation and waterlogging conditions were better in the ecotypes with effective salt and flood tolerance. We screened four vetiver ecotypes with good salt tolerance: Fiji; Nakhon Sawan and Monto (*C. nemoralis*); and Sri Lanka (*C. zizanioides*). Five vetiver ecotypes were screened with good flood tolerance: Maehae; Ratchaburi and Monto (*C. nemoralis*); Sri Lanka; and Songkhla 3 (*C. zizanioides*).

3.3 Nutrient contents

Under stress and non-stress conditions, all 19 vetiver ecotypes had storage ability of total N that was mainly in the shoots and was much lower than detected in the roots. In the control (non-stress condition), the storage ability of total N in the shoots was 1.12–1.7%. The Loei, Maehae, and Nakhon Sawan ecotypes had the highest storage ability, while Japanese had the lowest (Figure 3a). The storage ability of total N was reduced under stress conditions and all 19 vetiver ecotypes accumulated less than 1% total N. Prachuap Khiri Khan, Loei, and Songkhla 3 had high N storage in the shoots under saline water irrigation and Dyunrimtang stored the lowest amount (Figure 3b). Nangpaya, Maehae, and Fiji had high storage ability of total N under waterlogging conditions, while Syuntangbaimai had the lowest storage (Figure 3c). Our

Table 2. Average dry biomass (g) of 19 vetiver ecotypes grown under saline water irrigation and waterlogging conditions.

No.	Ecotype	Dry biomass (g)			
		Shoot		Root	
		Saline water irrigation	Waterlogging	Saline water irrigation	Waterlogging
1	Maehae	1.72±0.88ab	16.38±5.14ab	2.12±0.89ab	24.66±1.60ab
2	Maihuywai	0.60±0.41b	6.78±0.51ab	0.94±0.74b	11.16±0.77ab
3	Japanese	2.04±0.53ab	15.69±1.17ab	3.78±1.39a	25.52±1.33ab
4	Monto	2.03±0.58ab	19.71±2.75ab	3.60±1.01a	21.18±1.15ab
5	Prachuap Khiri Khan	1.48±1.31ab	6.01±0.68ab	1.52±0.58ab	11.02±1.57ab
6	Fiji	3.02±0.69a	7.43±1.03ab	1.90±1.32ab	9.62±1.24ab
7	Ratchaburi	1.62±0.39ab	23.36±1.46ab	1.90±0.69ab	31.87±2.83ab
8	Kamphaeng Phet 1	0.67±0.64b	-	1.68±0.53ab	-
9	Kamphaeng Phet 2	1.54±0.45ab	12.97±1.28ab	1.79±0.81ab	18.43±1.45ab
10	Sri Lanka	2.33±0.69a	27.58±2.77a	1.54±0.59ab	41.08±1.12a
11	Prarat Chathan	0.43±0.74b	3.60±0.34b	1.11±0.76b	5.83±0.08b
12	Roi Et	1.59±0.54ab	2.97±0.31b	1.51±1.20ab	3.60±0.33b
13	Loei	1.17±1.16ab	2.87±0.97b	1.61±1.10ab	1.82±0.05b
14	Nakhon Sawan	2.82±0.26a	6.17±0.47ab	1.59±0.21ab	11.69±0.23ab
15	Syuntangbaimai	1.99±0.88ab	2.43±0.07b	2.20±0.71ab	3.13±0.85b
16	Nangpaya	0.80±0.70b	2.74±0.01b	0.86±0.75b	3.14±0.09b
17	Surat Thani	0.96±0.90b	-	0.63±0.54b	-
18	Songkhla 3	1.05±0.29b	16.71±0.79ab	0.82±0.24b	18.81±0.65ab
19	Dyunrimtang	0.13±0.23b	2.09±0.07b	0.18±0.31b	2.78±0.06b

Values (mean±SD) in the same column with a common lowercase letter are not significantly different at the 0.05 probability level using LSD.

results validated the translocation ability of N from roots to shoots in non-stress conditions and also under stress conditions of saline water and waterlogging. ORDPB (1998) reported that the N content of one tonne of vetiver grass compost was as much as 43 kg of ammonium sulfate. Therefore, vetiver grass has the potential to be used as organic matter to increase soil fertility (Roongtanakiat, Chairoj, & Chookhao, 2000).

The Land Development Department (LDD, 1994) studied the content of plant nutrients in vetiver shoots and reported that the concentrations of N, P, and K were 2.5, 0.17, and 1.5%, respectively. Under non-stress conditions (control) the concentrations of N, P, and K were mainly in the shoots. The storage ability of total K in the shoots was the greatest at 2.02–3.14%. Roi Et, Japanese, and Prarat Chathan had high K stored in the shoots at 3.14, 3.13, and 2.94%, respectively, while Kamphaeng Phet 2 had the lowest storage (2.02%; Figure 3a). Total K in the roots was in the range of 0.38–1.15% and the greatest K concentration was in Roi Et (Figure 3d). Our study on the effect of salinity on nutrient uptake revealed that saline water increased total Na mainly in the shoots. The concentration of Na in the shoots was in the range of 0.77–2.57%. Prarat Chathan had high storage ability of Na in the shoots; however, high accumulation of Na affected plant growth and dry biomass, while Kamphaeng Phet 1 had the lowest Na storage ability (Figure 3b). Total P was contained in the shoots and roots at similar levels. On the other hand, total K had a higher concentration in the roots than in the shoots, which contrasted with the control (Figures 3b and 3e). In cucumber, saline water increased the Na in both the shoots and roots but decreased the K in the roots which resulted in a significantly reduced K/Na ratio in both the shoots and roots (Qing, Men, Gao, & Tian, 2017). In tomato, the accumulation of most nutrients, including K, Mg, P, and Cl, in the leaves was higher than in the roots but the accumulation of Na was higher in the roots compared to the shoots (Kahlaoui *et al.*, 2011). A study on the effect of salinity on nutrient uptake in cauliflower revealed that the concentrations of N, P, and K in the shoots and roots irrigated with saline water was lower than from irrigation using fresh water (Markovic, Markovic, Cerekovic, & Mihajlovic, 2013). The accumulation of more Na in the shoots of vetiver than in the roots would be of benefit in removing contamination of salt from soil or water as well as heavy metals, radionuclides, and organic pollutants (Menahem *et al.*, 2009; Mohammad *et al.*, 2018; Roongtanakiat & Akharawutchyanon, 2017). We selected the Parat Chathan ecotype with high Na uptake ability in the shoots for further study.

Vetiver grown under waterlogging conditions had lower concentrations of N, P, and K in the shoots than did the control (Figures 3a and 3c). A similar pattern in wheat and barley under waterlogging conditions was reported with decreased N, P, and K concentrations in the shoots compared to the control and this inhibited plant growth (Steffens, Hiitsch, Eschholz, Losak, & Schubert, 2005). Total N, K, and Na under submergence were accumulated mainly in the shoots with K having the highest storage ability at 1.32–2.17%. Prarat Chathan, Syuntangbaimai, and Fiji had high ability to store K in the shoots under waterlogging conditions (Figure 3c). Nevertheless, the total P concentration was higher in the roots and was in the range of 0.12–0.51%. The Japanese and Prarat Chathan ecotypes had the highest P concentrations which were approximately four-fold the control (Figure 3f). Under submergence, photosynthesis is limited because of the lower O_2 concentration in the soil. Delaune, Jugsujinda, and Reddy (1999) reported that the effect of oxygen stress in roots on P uptake by *Typha domingensis* suggested anaerobic treatments (no oxygen) had lower rates of P uptake compared to the oxidized treatment.

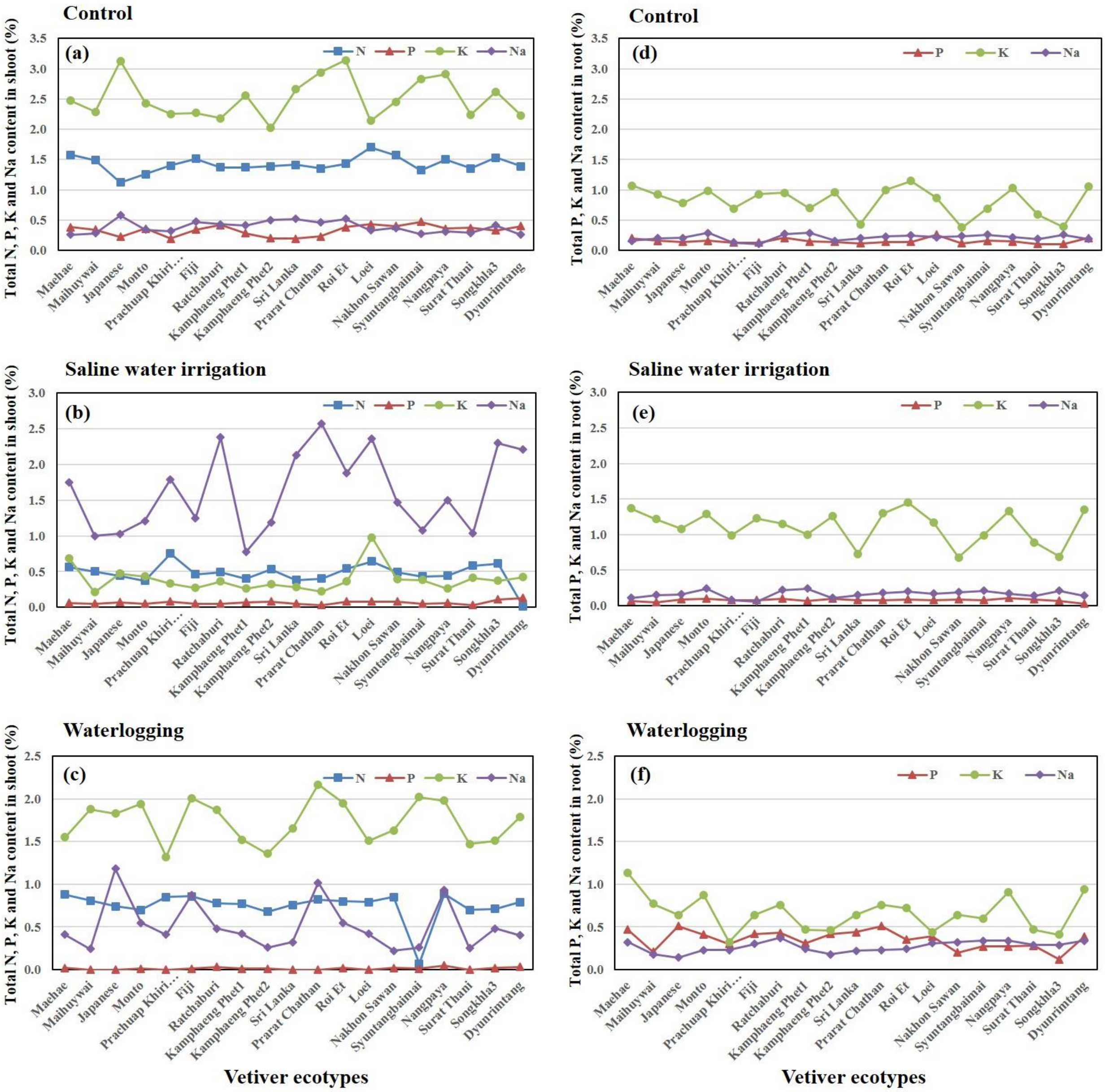

Figure 3. Plant nutrients of 19 vetiver ecotypes, total N, P, K, and Na contents in the shoots grown under (a) non-stress conditions (control), (b) saline water irrigation, (c) waterlogging and total P, K, and Na contents in the roots grown under (d) non-stress conditions (control), (e) saline water irrigation, (f) waterlogging.

4. Conclusions

The stress conditions applied to vetiver grasses resulted in decreased plant height, fewer numbers of new tillers, and lower dry biomass. The inhibition of vetiver growth may have been caused by low nutrient accumulation under the stress of saline water irrigation or waterlogging. Plant growth (height, tiller number, and biomass) exhibited beneficial characteristics which balanced the stress conditions and plant nutrient requirements for growth and development. We isolated eight profitable ecotypes with salt tolerance and flood tolerance. The Fiji, Nakhon Sawan, Sri Lanka, and Monto ecotpyes showed potential for salt tolerance while the Prarat Chathan ecotype had the highest translocation ability of Na from root to shoot. The Ratchaburi, Sri Lanka, Monto, Maehae, and Songkhla 3 were flood tolerant. Those desirable ecotypes are valuable for further vetiver improvement using gamma mutagenesis and screening for salt tolerance and flood tolerance in our breeding program.

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