CHAPTER 3

Results and discussion

The figures of PT1 rice plants after transplanting were shown in Figure 3.1. From Figure 3.1 (a) the rice plants on 17 days after transplant shows 9 rows of experiments and 5 column of replicates. The increasing of number of tillers could be seen in Figure 3.2 (b). The rice plants at reproductive and ripening stage were shown in Figure 3.2 (c), (e)-(f), respectively. Figure 3.2 (g) shows the rice plant at the harvest day. It could be observed that the rice plants hydroponically grown using different concentrations of N fertilizer and levels of salinity stress exhibited different growth rates (Figure 3.1 (d)). The extreme level of salinity had a negative impact on the rice plants. The salt-stressed plants (Treat. 1, 4 and 7) which appeared relatively small and had slightly fewer tillers, less root mass, shorter, thinner and chlorotic leaves compared to the less- or non-salinized condition plants. However, the roots of the rice plants with the imposition of the high salt stress had longer length during the early of growth period. The electrical conductivities (EC) of the nutrient solutions for the Treat. 1, 4 and 7 (7.42, 6.94 and 7.99 $S.cm^{-1}$, respectively) were high due to the amount of the salt added according the design. Relatively high value of the EC parameter in Treat. 1 was due to the contribution of the high concentration of NH_4NO_3 in the solution.

The better development of the rice plants from Treat. 2 and 5 were probably due to the high concentration of nitrogen. Interestingly, although the nitrogen concentrations in Treat. 3 and 8 were relatively low controlled by the design, the reduction in growth of the plants was not clearly observed. This could be that the plants were not much affected by the severe salt-stress and implied that the nitrogen contents in the nutrient solution were enough for the rice cultivation.

(a) 17 days

(b) 35 days

(c) 82 days

(d) 75 days

(e) 103 days

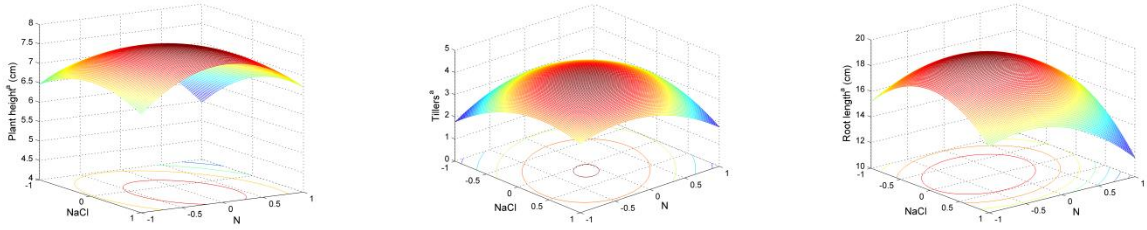
(f) 109 days

(g) 125 days

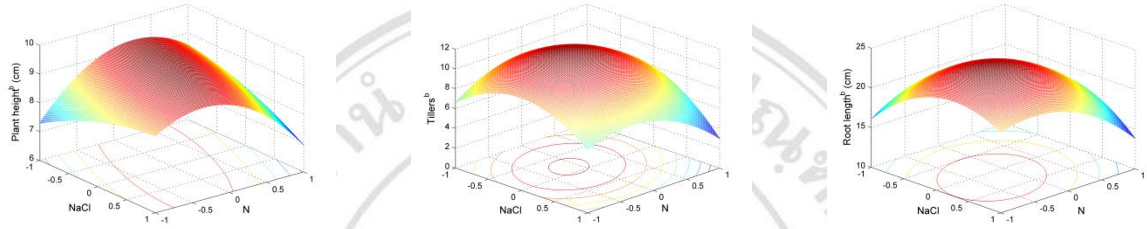
Figure 3.1 The rice plants after the transplanting

3.1 PLS prediction and coefficients

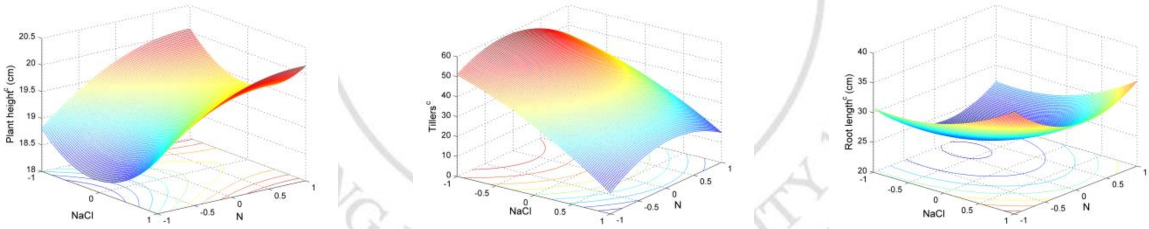
It is possible to use each of the plant growth parameters and yield components as a response for the experimental data. A conventional approach is to fit the data with a second-order polynomial model to obtain regression coefficients [29]. The analysis of variance (ANOVA) could be carried out to evaluate model adequacy and determine the statistical significance of the regression coefficient terms. The fitness of the equation to the responses can be estimated using coefficient of determination (Q^2). The results of these analyses were conducted using Design Expert trial version 10.0 (Stat-Ease, Inc.) and shown in the supplementary Table 3.1 and Table 3.2. In addition, the interaction effects of the studied factors on the recorded parameters were investigated using response surface methodology (RSM) [41] and visualized in Figure 3.2 and Figure 3.3.



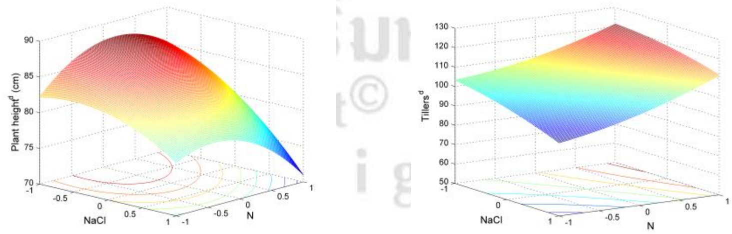
(a) Plant height^{a} (b) Tillers^{a} (c) Root length^{a}



(d) Plant height^{b} (e) Tillers^{b} (f) Root length^{b}

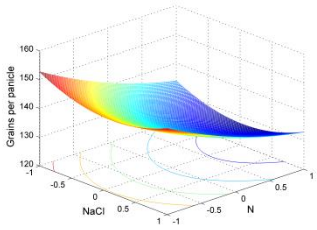


(g) Plant height^{c} (h) Tillers^{c} (i) Root length^{c}

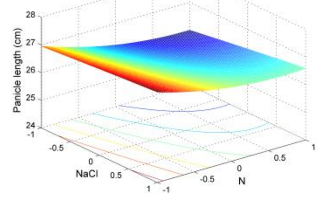


(j) Plant height^{d} (k) Tillers^{d}

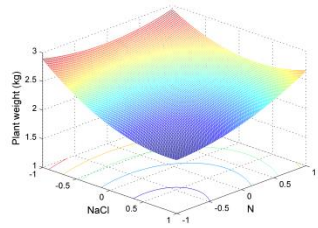
Figure 3.2 Response surface contour plots showing interactive effect of N and Na concentrations on the growth parameters. Superscript *a* = transplanting, *b* = tillering, *c* = panicle initiation and *d* = harvest.



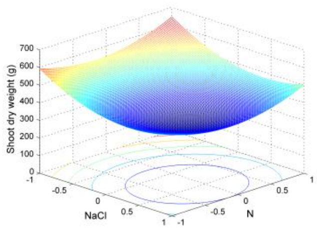
(a) Grains per panicle



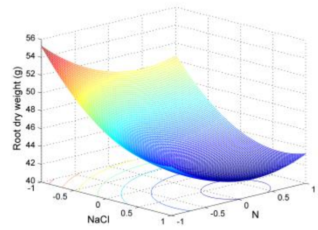
(b) Panicle length



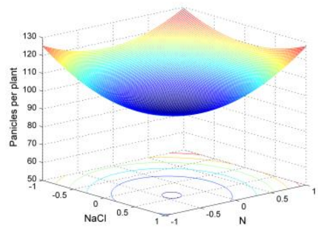
(c) Plant weight



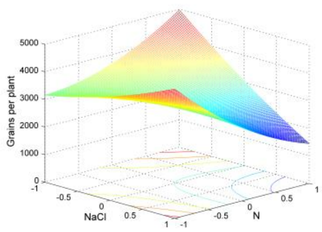
(d) Shoot dry weight



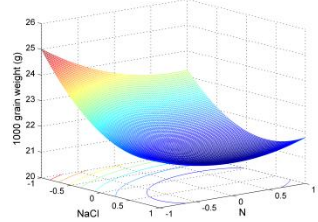
(e) Root dry weight



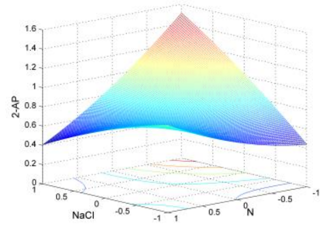
(f) Panicles per plant



(g) Grains per plant



(h) 1000 grain weight



(i) 2AP

Figure 3.3 Response surface contour plots showing interactive effect of N and Na concentrations on the yield-related components.

Table 3.1 Regression coefficients, coefficient of determination (R^2) and F-test values of the predicted second-order polynomial models for the growth parameters.

Regression coefficients	Plant height^{a}	Tillers^{a}	Root length^{a}	Plant height^{b}	Tillers^{b}	Root length^{b}	Plant height^{c}	Tillers^{c}	Root length^{c}	Plant height^{d}	Tillers^{d}
Intercept	7.65	3.60	18.69	9.37	10.56	23.03	19.13	44.37	25.09	85.00	105.25
N	-0.15	-0.16	-0.66	-0.06	-0.48	-1.09**	0.41*	0.61	-1.29**	-0.37	7.17**
Na	0.24	0.04	-0.50	-0.03	-0.72**	0.38	0.05	-11.43***	2.23***	-3.00***	-4.62
N×Na	0.17	-0.12	-0.23	-0.35	-0.38	-0.61	-0.01	2.65	0.00	-0.71	0.16
N^2	-0.44	-0.28	-0.88	-0.60**	-0.89*	-1.40*	-0.15	-4.53*	2.37***	-1.71	1.23
Na^2	-0.23	-0.28	-1.25	-0.12	-1.07**	-1.70**	0.33	-1.57	1.21	-1.02	-1.09
F-value (model)	1.24	1.13	1.04	1.74	2.77**	2.26*	1.25	9.73***	7.46***	2.66**	2.06*
F-value (lack of fit)	1.09	0.89	0.98	0.32	4.22**	1.50	0.15	11.02***	6.31***	5.16***	0.28
R^2	0.15	0.14	0.13	0.20	0.28	0.24	0.15	0.58	0.52	0.28	0.23

Superscript *a* = transplanting, *b* = tillering, *c* = panicle initiation and *d* = harvest. Levels of significances $^{*}p < 0.10$, $^{**}p < 0.05$ and $^{***}p < 0.01$.

Table 3.2 Regression coefficients, coefficient of determination (R^2) and F-test values of the predicted second-order polynomial models for the yield components.

Regression coefficients	Grains per panicle	Panicle length	Plant weight	Shoot dry weight	Root dry weight	Panicles per plant	Grains per plant	1000 grain weight	2AP
Intercept	133.96	26.46	2.18	379.13	43.33	101.59	2939.52	21.33	0.77
N	-6.08**	-0.27	0.12**	15.73	-1.27	4.50*	-203.14	-0.29	-0.10***
Na	-0.32	0.18	-0.23***	-50.52***	-3.10***	-3.81	-297.31	-0.78**	0.12***
N×Na	0.93	0.05	0.07	-4.21	0.17	2.24	-524.94	0.29	-0.17***
N^2	1.54	0.13	0.07	37.52**	1.07	5.82	259.74	0.31	0.05
Na^2	2.88	-0.01	0.13*	45.59**	1.48	4.72	128.60	0.54	-0.01
F-value (model)	0.97	0.40	7.29***	5.91***	2.11*	1.58	0.95	1.58	18.23***
F-value (lack of fit)	0.47	0.53	1.27	0.53	0.23	0.85	1.41	0.66	-
R^2	0.12	0.05	0.51	0.46	0.23	0.18	0.12	0.18	0.72

Levels of significances $^{*}p < 0.10$, $^{**}p < 0.05$ and $^{***}p < 0.01$.

Based on the planting condition, growth and yield-related data, a PLS model was established. Using leave-one-out cross-validation (LOOCV), the number of the optimum PCs of PLS modeling was 2 PCs which accounted for 95.79% of the ***X*** data variance and 93.39% of the ***y*** variance. Table 3.3 shows the predictive results of the 2AP concentrations in the PT1 rice grains for each of the experimental treatments. The correlation graph between the observed and predicted 2AP values was shown in Figure 3.4.

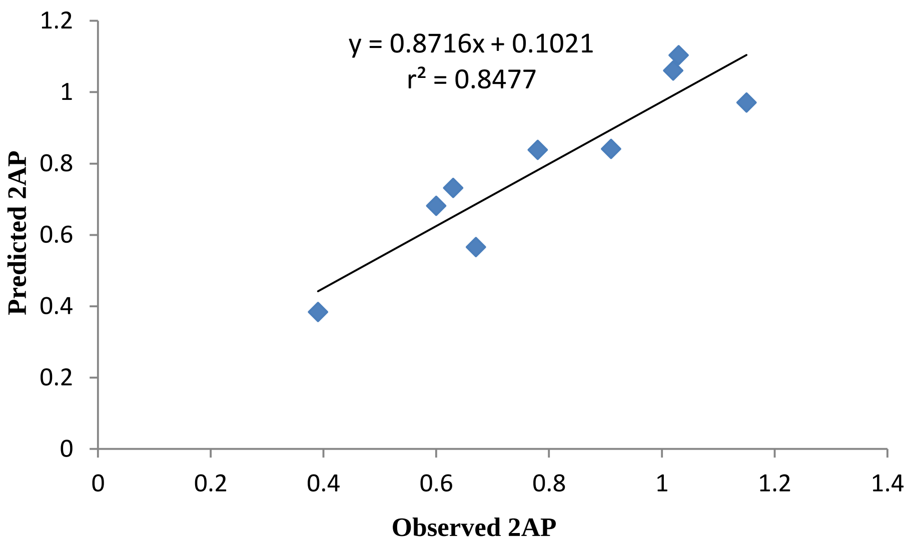


Figure 3.4 A correlation graph between predicted and observed 2AP values.

Table 3.3 Observed and predicted 2AP concentrations (ppm) in the rice grains using PLS

Treatments	2AP (ppm)	
	Observed	Predicted
1	0.39	0.38
2	0.63	0.73
3	0.60	0.68
4	1.15	0.97
5	0.91	0.84
6	1.03	1.10
7	1.02	1.06
8	0.67	0.57
9	0.78	0.84
Q^2		0.848
RMSECV		0.091

The cross-validated explained variance (Q^2) was 0.8470 with the root mean square error of cross-validation (RMSECV) value of 0.091. The PLS coefficients and variable influence on projection (VIP) values for each of the parameters were shown in Table 3.4. It is necessary to note that the PLS coefficients imply the importance of the parameters with respect to the prediction of the 2AP contents. The magnitudes of the coefficients can be used to determine how much the variables are significant or influential on the prediction model. The greater the magnitude is, the more important it is likely to be associated with the 2AP contents. The sign of the coefficient can be used to indicate the direction of the effect, for example, positive (+) value indicates a direct proportion relationship with the response and vice versa. In Table 3.4, the parameters are ranked from the best to the worst fits according to the sizes of their coefficients.

To determine whether the observed parameters were truly significant and thus important to the 2AP contents in the rice grains, the empirical method was used [19]. The PLS coefficients were compared with the background distribution generated from the randomly permutated 2AP vectors (Figure 3.5). The significance for each studied parameter was included in Table 3.4 showing that Na, the rice yield, the shoot dry weight, the number of tillers per plant and N were significant with an empirical significance of 90%. These parameters were selected and discussed in details later in the following section.

Table 3.4 PLS coefficients and VIP values for the design, growth parameter and yield component data

Parameter names	PLS coefficients	VIP values
Na	0.2033***	2.4532
Grains per plant	0.1539**	3.2420
Shoot dry weight	-0.1489**	1.7208
Tillers^{c}	-0.0802*	1.0658
N	-0.0722*	0.7470
Tillers^{d}	-0.0419	0.4547
EC	0.0372	0.4545
Panicles per plant	-0.0325	0.3676
Root length^{c}	0.0242	0.2837
Root dry weight	-0.0224	0.2708
Root length^{b}	0.0148	0.1532
Grains per panicle	0.0138	0.1419
Plant weight	-0.0117	0.1296
Plant height^{d}	-0.0085	0.1460
Tillers^{a}	0.0077	0.0869
Plant height^{a}	0.0061	0.0720
1000 grains weight	-0.0046	0.0611
Panicle length	0.0039	0.0428
Tillers^{b}	-0.0034	0.0961
Plant height^{c}	-0.0027	0.0280
Plant height^{b}	0.0014	0.0185
Root length^{a}	0.0013	0.0562

Superscript *a* = transplanting, *b* = tillering, *c* = panicle initiation and *d* = harvest. Asterisks indicate significant correlation at $^{*}p < 0.10$, $^{**}p < 0.05$ and ***p < 0.01

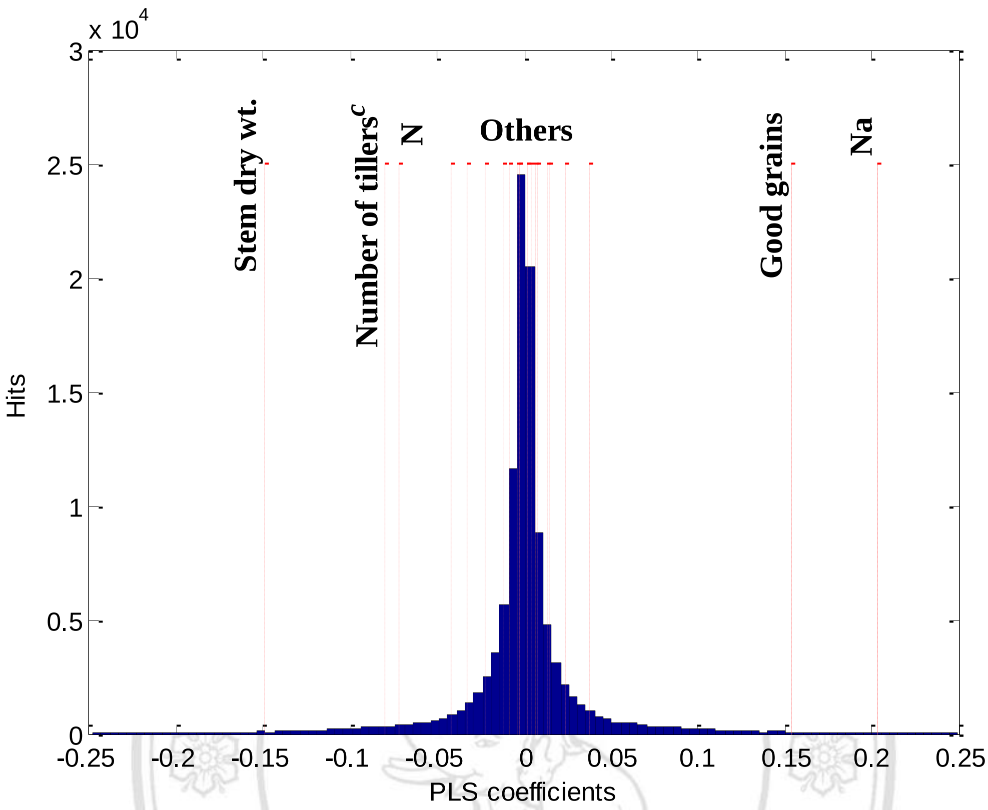


Figure 3.5 A null distribution for confirming the significance of PLS coefficients. The red dotted lines indicate the coefficients of the studied parameters

3.2 SSOM analysis

3.2.1 Exploratory analysis using supervised color shading maps

SOM is an adaptive learning model used to imitate the structure of training samples so that the variation of the training samples could be reserved in the trained map. Figure shows supervised color shading maps where each of the BMUs was labeled according to the samples' name. When a trained map is visualized using supervised color shading maps, each map unit is shaded according to the nearest class. In this research, although the class membership or the response data was included in the same way as in the supervised SOM, it was not used when locating the BMUs of the training samples, because the supervised SOM was simplified for an exploratory data analysis and to avoid over fitting of the trained map.

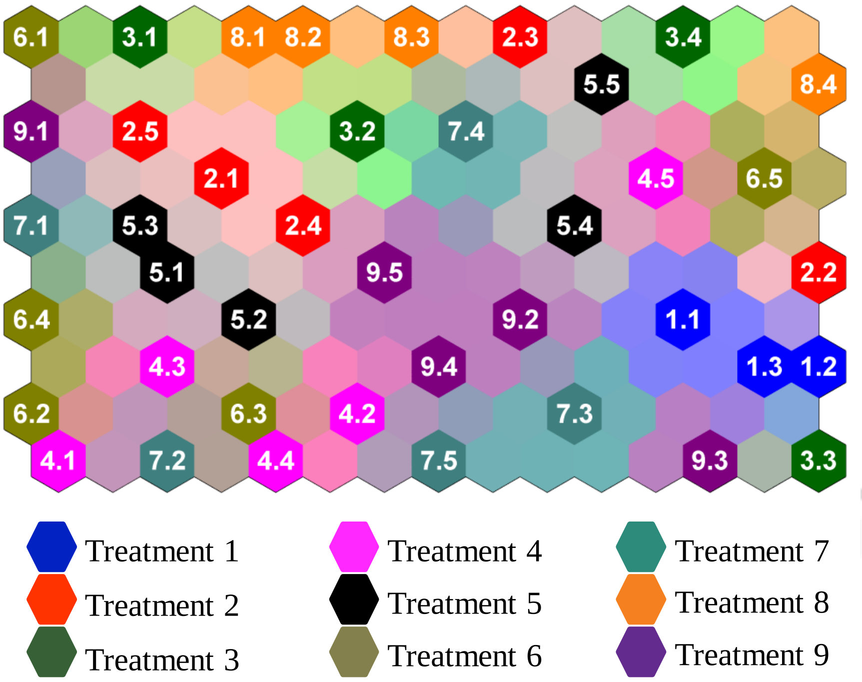


Figure 3.6 Supervised color shading map. The BMUs are labeled based on the treatments and corresponding replication numbers.

Using a supervised color shading map, some clusters in the map can be observed. Samples from the same treatment were located on the same area on the map, for example, the sample no. 1.1, 1.2 and 1.3 on the lower-right and the sample no. 9.2, 9.4 and 9.5 in the middle of the map. However, some of them were apart from the group, for example, the sample no. 9.1 and 9.3 which were on the upper-left and lower-right of the map, respectively. This reflected the variation of the studied samples. The CCD used in this work designed a set of the experiments in a discrete manner expecting that studied factors were orthogonal. If the design data was only used, the samples would be ideally clustered into 9 different regions corresponding to the 9 treatments.

3.2.2 Component planes

It is possible to display a component plane of the trained map for each parameter, separately, which are somewhat analogous to loadings in principal component analysis (PCA). Component planes can be used to see how each parameter influences the map and which samples a parameter is the most likely associated with. Figure 3.7 (a) shows the response profile (a component plane of the response) visualizing the 2AP values of the supervised training map. The component planes for the parameters namely N (Figure 3.7 (b)), Na (Figure 3.7 (c)), number of tillers during the panicle initiation stage (Figure 3.7 (l)), shoot dry weight (Figure 3.7 (r)) and yields (Figure 3.7 (u)) are also shown.

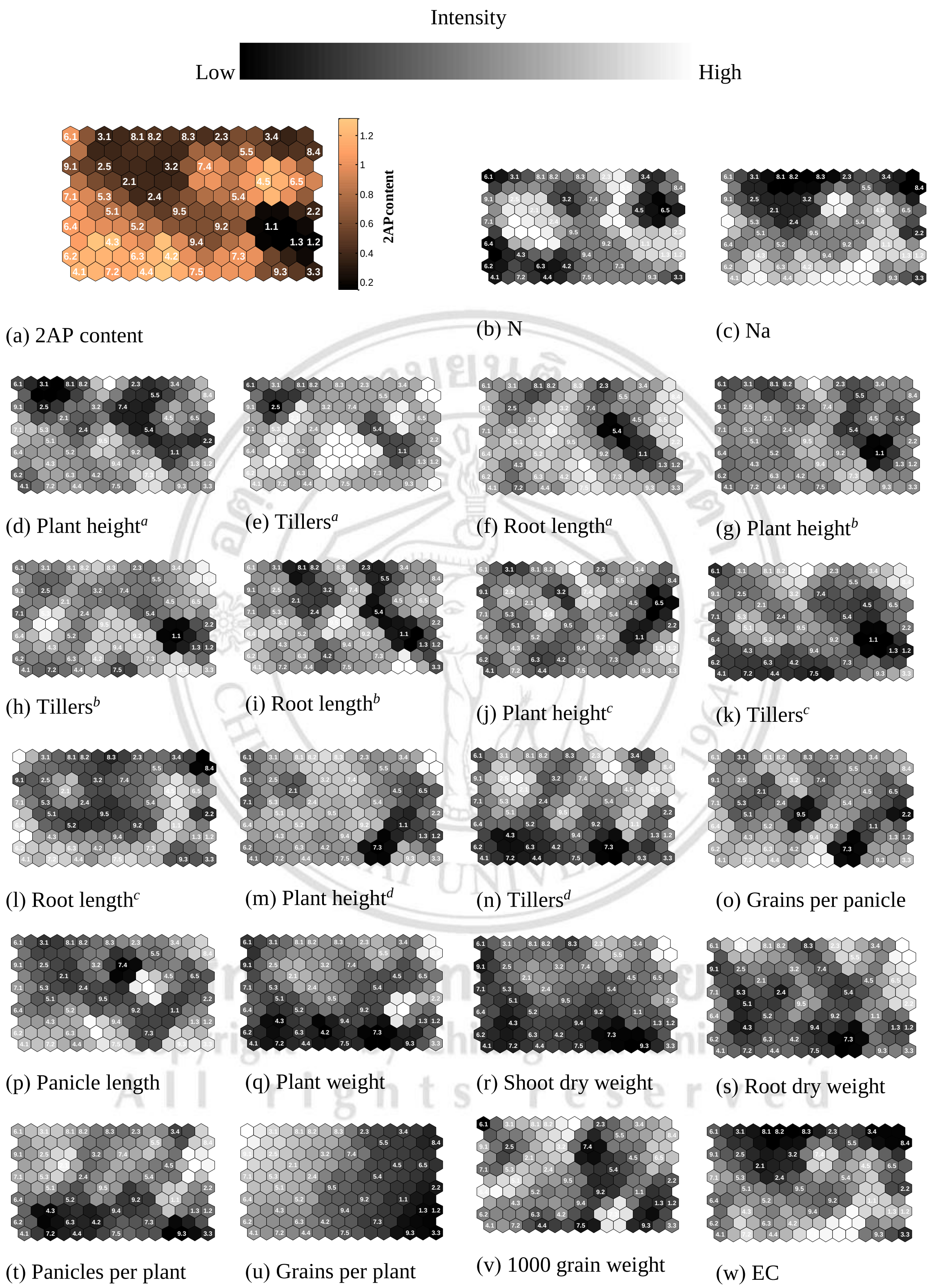


Figure 3.7 (a) a response plane of the 2AP values and (b)-(w) component planes for each of the parameters (*a* = transplanting, *b* = tillering, *c* = panicle initiation and *d* = harvest).

3.3 Effects of salt stress and nitrogen fertilizer

Salt stress and nitrogen fertilizer were among the parameters of interest which may affect the 2AP contents in rice grains. Previously, some researcher groups reported that the salt stress had positive impact on the rice grain flavor [8,9], while some other researchers were interested in the effect of nitrogen in soil on the 2AP content in rice grains [11]. However, the communal effect of Na and N on the 2AP content was not mentioned in those previous reports. In Table 3.4, the PLS coefficient of sodium salt was the largest and positive (+) value. Therefore, this parameter was expected to be important and directly proportional to the 2AP content in grains implying that the increase in the salt stress could enhance the rice aromatic quality. This could be due to the fact that the salinity led to ionic imbalance and toxicity and it also caused osmotic stress [42]. In response to the osmotic stress in plants, the biosynthesis of proline, an amino acid that functioned as an osmotic adjustment, was promoted [43]. This amino acid was the main precursor of 2AP [5]. Therefore, if the concentrations of the salt in the solutions were sufficient to induce the osmotic stress, the rice grains with high level of 2AP content were likely yielded. Nitrogen had a smaller value of the coefficient so it influenced less on the 2AP content (P-value = 0.0751) and the negative coefficient implied that this parameter did not contribute towards the 2AP content in the rice yields. Nevertheless, the effect of nitrogen applied could be suppressed due to the presence of Cl^- that reduced NO_3^- uptake in plants [44].

When compared with an interpretive display of the component plane visualization of the Na concentration (Figure 3.7 (b)), 2AP values (Figure 3.7 (a)) were increased by increasing of Na concentrations. However, despite the high concentration of the sodium salt, the achieved 2AP content was low as shown at the lower right of the map (the region of the treatment no. 1). This component plane visualization implied that, in the presence of salt stress (high amount of NaCl), boosting the concentration of N could dramatically decrease the 2AP content in the rice grains.

3.4 Relationship between grain yield, shoot dry weight and numbers of tillers and 2AP contents

Based on the PLS coefficient, the number of grains per plant had a positive effect upon the aromatic quality. This result, in relation to the negative coefficient of thousand grain weight, explained why the rice grains with high aromatic quality generally have a smaller grain size [8]. The number of tillers during the panicle initiation stage and shoot dry weight per plant after the harvest were inversely correlated to the 2AP content. The reason could be that the salt stress disturbed the growth of the rice plants. The reduction in the plant biomass was possibly due to the decrease in carbohydrate accumulation caused by reduction in carbon assimilation [45]. In fact, the salt stress could affect to the other growth and yield-related parameters; however, they were not significant in relation to the change of the 2AP content in the rice grains. Otherwise, similar patterns in the components planes to the response should be observed. Less clear pattern for the component plane of the number of tillers (Figure 3.7 (k)) could be due to the fact that this parameter had a smaller size of the PLS coefficient but this parameter could contribute to the increase in the grain yield parameter nevertheless.

The PLS coefficients could show the effect of each studied factors on response factor, however the component planes of SSOM could imply more detail than PLS coefficients. The different between these two techniques is PLS is the linear method but SOM is the non-linear method. Due to the complicate and non-linear relations, SOM could be better to investigate them.