

# Estimation of Nitrogen Content and Marketable Yield Using Chlorophyll Readings

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**Abstract:** Accurate estimation of nitrogen content and yield is crucial and these can be predicted through chlorophyll readings as indicators. To explore the diagnosis model, a field experiment was conducted in a greenhouse at Yang ling, northwest China. The SPAD values results estimated the nitrogen status, total marketable yield, and rates of reduction in market yield. There was a positive correlation between SPAD value at the middle leaf (MD-SPAD) and nitrogen accumulation, and between SPAD value under the leaf (UD-SPAD) and nitrogen accumulation ( $R^2=0.37-0.78$ ,  $P<0.01$ ,  $n=27$ ). The MD-SPAD and UD-SPAD were positively correlated with total marketable yield during the growth period ( $R^2=0.43-0.59$ ,  $P<0.01$ ,  $n=27$ ). However, a poor correlation was observed at the upper leaf (UP-SPAD) in the expanding fruit period but was better in the mature period. Moreover, the nitrogen nutrition index (NNI) and MD-SPAD were positively correlated ( $R^2=0.42-0.55$ ,  $P<0.01$ ,  $n=54$ ). There SPAD values and reduction rates of ranks were positively correlated in marketable yield ( $R^2=0.48-0.52$ ,  $P<0.01$ ,  $n=54$ ), including middle fruit yield (MFY) and big fruit yield (BFY). This was similar between SPAD values and reduction rates of total marketable yield ( $R^2=0.50$ ,  $P<0.01$ ,  $n=54$ ). This indicated that nitrogen accumulation estimates including nitrogen accumulation in stem, leaf, fruit, and the overall nitrogen accumulation, as well as total marketable yield, is not accurate via UP-SPAD during fruit expanding period but it is opposite in mature period. When simultaneously considering estimation of nitrogen accumulation, NNI and marketable yield using chlorophyll readings, which were adopted by mean of SPAD values the different leaf positions or SPAD values at the middle leaf. On the other hand, UP-SPAD should be cautiously used to estimate nitrogen status, yield, and yield reduction rates. The overall marketable yield was optimal under W2 of 2488 m<sup>3</sup>hm<sup>-2</sup> and N2 of 300 kg hm<sup>-2</sup>. The reduction rates during middle fruit yield, big fruit yield, and total marketable yield ranged from 31%-38%. The big fruit yield was the highest, indicating that the big fruit yield is highly affected by severe water stress in the blossom and fruit period.

**Keywords:** Nitrogen Status, SPAD Value, Relationship, Leaf Position, Marketable Yield

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## 1. Introduction

Recently, expansion of vegetable planting in greenhouse, uncontrolled irrigation and use of nitrogen fertilizers have led to waste of water resources and environmental pollution [1, 2]. Appropriate irrigation and application of the right amount of nitrogen fertilizer could improve yield, nitrogen uptake and efficiency of water use in crops such as tomato [3, 4], wheat [5, 6], and cucumber [7, 8]. Therefore, type of irrigation and amount of nitrogen fertilizer used could greatly increase production and nitrogen uptake in crops. Chlorophyll meter

reading, to some extent can assess the level of nitrogen and the resultant yield in crops such as rice [9] and potato [10]. Chlorophyll content can be rapidly measured using chlorophyll meter, a simple and universal diagnostic instrument. Studies have revealed a good relationship between chlorophyll, nitrogen uptake and yield [11, 12]. Nitrogen nutrition index in crops has been estimated by chlorophyll readings [13-15]. However, for an accurate model on the basis of estimation of nitrogen content and yield by chlorophyll readings, relationships of chlorophyll content in different growth periods with nitrogen status and yield may exhibit a dynamic change. A previous study showed a linear relationship between

chlorophyll and concentration of leaf nitrogen [16], but study also showed a index relationship between chlorophyll content and nitrogen accumulation [17]. The difference in the relationships may be due to non-uniform selection standard of leaf positions during chlorophyll measurement. Previous studies focused on selection of fully expanded leaf [12, 18-20], whereas it does not have uniform position or detailed description. It has been shown that Chlorophyll sensitivity of frond is based on age. Generally, the classification accuracy tends to decrease as frond gets older [21], which may depict dynamic change of chlorophyll content in young or old leaf. The upper one-third of the plant proved to be a reliable tissue source in five specialty cut flowers, generally, young leaf samples collected early in the season had higher nutrient concentrations as compared with the same leaf position at mid or late season [22], which indicates that physiological metabolism and nutrient status could be affected by different leaf position or growth period. Selection of leaf position and growth period could be particularly important when dealing with evaluated accuracy of nitrogen level with chlorophyll readings. Chlorophyll changes in different growth period, thus the accuracy of the relationship between chlorophyll and nitrogen level or yield may also change. It has also been shown that the relationship of chlorophyll, in the expansion period or mature period, with nitrogen nutrient index and yield are stronger than in seedling stage [23]. Thus, the chlorophyll in different stages of growth could have a significant impact on the assessment of nitrogen level and yield. Thus, the relationship of chlorophyll with level of nitrogen and with yield could be more accurate if chlorophyll measurement is divided in different growth stages or leaf positions.

The objectives of this study were: (1) to investigate the effects of level of irrigation and amount of nitrogen fertilizer applied on chlorophyll readings, nitrogen content and marketable yield (2) to estimate nitrogen content, marketable yield and reduction rate in marketable yield with chlorophyll readings at different positions in stages of growth. (3) To quantify reduction rates of ranks in marketable yield due to water stress during critical growth period.

## 2. Materials and Methods

### 2.1. Experimental Site and Materials

This study was conducted in a greenhouse at an

Agricultural Research Farm of the Agricultural Cooperative in Yang Ling, Shaanxi province, China (latitude 34°17'N, longitude 108°10'E, altitude 527m). The study area has a typical semi-arid climate with an average annual rainfall of 632 mm, sunshine of 2163.8 h and evaporation of 1500 mm. The greenhouse of 40 m long and 8 m wide with total planting area  $38 \times 7 = 266 \text{ m}^2$  had an east-west orientation, with crop rows aligned north-south. Tomato plants (Kai de dong guan) were transplanted on 02-02-2019 and uprooted on 03-07-2019 during the winter-spring season. Tomato seedlings were evenly transplanted along each edge of the raised bed which was 7m long and 0.9m wide. The planting spacing and row spacing were 0.35m and 0.50m, respectively. Surface drip irrigation was applied via plastic film mulching. Each dripper had a flow rate of  $2.1 \text{ L} \cdot \text{h}^{-1}$  and spacing were 0.35m apart. Heavy loam soil with the bulk density of  $1.40 \text{ g} \cdot \text{cm}^{-3}$  and field water capacity of  $0.24 \text{ cm}^3/\text{cm}^3$  in the 0-60 cm was used. The available nitrogen, phosphate and total potassium fertilizer content in 0-40cm soil layer before planting were  $29.69 \text{ mg} \cdot \text{kg}^{-1}$ ,  $21.51 \text{ mg} \cdot \text{kg}^{-1}$ , and  $174.35 \text{ mg} \cdot \text{kg}^{-1}$ , respectively.

### 2.2. Experiment Design

Three levels of nitrogen and three levels of irrigation were applied. The three nitrogen fertilizer (N) levels were set as: lower nitrogen (N1) of  $150 \text{ kg}/\text{hm}^2$ , moderate nitrogen (N2) of  $300 \text{ kg}/\text{hm}^2$  and high nitrogen (N3) of  $450 \text{ kg N}/\text{hm}^2$  as conventional nitrogen fertilizer application, N1 and N2 were a reduction of 66% N3 and 33% N3, respectively. The three levels of irrigation were set as: severe water stress (W1) of 50%W3 in blossom and fruit period, mild water stress (W2) of 75% W3 in blossom and fruit period, full irrigation (W3) of 75% -90% in field water capacity (75% as the lower limit and 90% as the upper limit of field water capacity, respectively) in whole growth period. When the average of soil moisture content in 0-60cm soil layers was 75% water field capacity, irrigation of the plants was started. The frequency of irrigation and time were the same as those of control treatment. A completely random design was used in this study, with 9 treatments, namely: W1N1, W1N2, W1N3, W2N1, W2N2, W2N3, W3N1, W3N2, W3N3, and each was conducted in triplicate. The details are provided in Table 1 and Table 2.

Table 1. Details of 9 treatments: nitrogen fertilizer application rates and irrigation level rates.

Treatments	N rates ( $\text{kg} \cdot \text{hm}^{-2}$ )	Rates of irrigation levels		
		Seedling period	Blossom and fruit period	Mature period
W1N1	150	W3	50%W3	W3
W1N2	300	W3	50%W3	W3
W1N3	450	W3	50%W3	W3
W2N1	150	W3	75%W3	W3
W2N2	300	W3	75%W3	W3
W2N3	450	W3	75%W3	W3
W3N1	150	W3	W3	W3
W3N2	300	W3	W3	W3
W3N3	450	W3	W3	W3

According to the local traditional fertilizer supply, amount of phosphorus fertilizer (P) and potassium fertilizer (K) were 200 kg·hm<sup>-2</sup> and 360 kg·hm<sup>-2</sup>, respectively. 100% P, 40% K and nitrogen fertilizer of 100 kg·hm<sup>-2</sup> were applied into the tillage as the base fertilizer. The rest of K and N were evenly applied with irrigation water in blossom and fruit period. Nitrogen, phosphorus and potash fertilizer were urea (≥46%), Superphosphate (P<sub>2</sub>O<sub>5</sub>: p≥16%), and potassium sulfate (K<sub>2</sub>O:

≥52%), respectively. The level of irrigation of each treatment was controlled by water meter. The level of irrigation was 22 mm on transplanting date and no irrigation was needed for a long time after transplanting to provide a conducive environment for root development. The experimental treatments were carried out after the survival of tomato seedlings and mulch films were placed in the north-south direction before transplanting.

Table 2. Details of the study: level of irrigation and nitrogen fertilizer application.

Irrigation date	Growth periods	Level of irrigation (m <sup>3</sup> ·hm <sup>-2</sup> )			Nitrogen fertilizer application (kg·hm <sup>-2</sup> )		
		W1	W2	W3	N1	N2	N3
2019.01.26		\	\	\	100	100	100
2019.02.02	Seedling period	221.19	221.19	221.19	0	0	0
2019.02.22		221.19	221.19	221.19	0	0	0
2019.03.05		110.6	165.89	221.19	0	0	0
2019.03.19	Blossom period	110.6	165.89	221.19	12.5	50	87.5
2019.03.29		110.6	165.89	221.19	0	0	0
2019.04.06		110.6	165.89	221.19	12.5	50	87.5
2019.04.17	Fruit expanding period	110.6	165.89	221.19	0	0	0
2019.04.29		110.6	165.89	221.19	12.5	50	87.5
2019.05.10		110.6	165.89	221.19	12.5	50	87.5
2019.05.23	Mature period	221.19	221.19	221.19	\	\	\
2019.06.01		221.19	221.19	221.19	\	\	\
2019.06.09		221.19	221.19	221.19	\	\	\
2019.06.18		221.19	221.19	221.19	\	\	\
Whole growth period		2101.34	2488.37	2875.47	150	300	450

### 2.3. Measurements

#### 2.3.1. Calculation of Nitrogen Content

Biomass accumulation of tomato was determined using 9 samples per treatment in two growth stages, including fruit expanding period (55DAT-105DAT), samples destructed at 71DAT and 104DAT, and mature period (106DAT-153ADT), samples destructed at 129DAT and 150DAT, DAT refers to days after transplanting. Fresh plants were divided into several parts including root, stem, leaf and fruit, heated for 30 min at 105°C to halt metabolic processes then dried at 75°C until they attained a constant weight. Dry matter of different parts including; root, stem, leaf, fruit, and total dry matter were determined. The samples were then stored for further chemical analysis. Nitrogen concentration was determined using a flowing analyzer AA3. Nitrogen (N) accumulation at 150 DAT was calculated using equation 1 and 2 [17]:

$$NA_j = DM \times NC_j \quad (1)$$

$$TNA = \sum NA_j \quad (2)$$

Where NA, DM, NC, and TNA are the nitrogen accumulation in parts of plant, dry matter in parts of plant, nitrogen concentration in parts of plant and total nitrogen accumulation at 150DAT, respectively, j represent root, stem, leaf and fruit at 150DAT.

#### 2.3.2. Calculation of Nitrogen Nutrition Index (NNI)

Nitrogen nutrition index (NNI) can directly reflect the nutrient status of nitrogen in crops, and can be calculated as shown in equations 3 and 4 [24]:

$$\%N_c = a_c \cdot DW_{\max}^{-b} \quad (3)$$

$$NNI = N_t / N_c \quad (4)$$

Where; %N<sub>c</sub> is the critical nitrogen concentration value, *a<sub>c</sub>* is the critical nitrogen concentration value when the over ground biomass is 1 t/hm<sup>2</sup>, DW<sub>max</sub> is the maximum value of overground biomass (t/hm<sup>2</sup>), b is statistical parameter which determines the slope of dilution curve in the critical nitrogen concentration, N<sub>t</sub> is the measured value of nitrogen concentration (g/100g), and N<sub>c</sub> is the nitrogen concentration value according to dilution curve model in the critical nitrogen concentration when overground biomass is the same. When NNI=1, the nutrient status is optimum; When NNI>1, the nutrient status is excess; When NNI<1, the nutrient status is deficient.

#### 2.3.3. Measurement of SPAD Value

Chlorophyll content of leaves is generally measured using SPAD meter. In this study, the leaves were divided into; under leaf consisting of 11th, 12th and 13th, middle leaf consisting of 14th, 15th and 16th, and upper leaf consisting of 17th, 18th and 19th from the bottom to the up. To prevent plant diseases, the leaves (1-9th) were disposed in blossom and fruit period (78DAT) for underside ventilation according to local management. Samples were taken every 14-19 days. The SPAD values were determined using an average of 9 samples per treatment in the growth periods, including fruit expanding period, samples destroyed at 70DAT, 89DAT and 103ADT, and mature period, as well as samples destroyed at 119DAT 133DAT and 149DAT. 9 plants were randomly selected for

each measurement per treatment. Each leaf had three SPAD values including, one value around the midpoint of the leaf and two values around the sides of the midrib, all SPAD values were averaged [25].

**2.3.4. Measurement of Marketable Yield**

Marketable yield was determined using the average of tests done in triplicate per treatment of 9 tomato plants. Each picking yield was separated in different ranks of yield based on single fruit weight, determined via a 0.01 g precision electronic balance. After all the picking was done, different ranks of yield were determined. According to the investigation of purchase of tomato in the local market, they were divided into three categories based on single fruit weight: 60 g ≤ w < 125 g (F<sub>60-125</sub>), 125 g ≤ w < 250 g (F<sub>125-250</sub>) and 250 g ≤ w < 350 g (F<sub>250-350</sub>). Thus, the ranks of marketable yield were; small fruit yield (F<sub>60-125</sub>), middle fruit yield (F<sub>125-250</sub>) and big fruit yield (F<sub>250-350</sub>), and sum of these yields is the total marketable yield. Reduction rate of different ranks in marketable yield is calculated as:  $(V_{mi} - V_i) / V_{mi} \times 100\%$ , where;  $V_{mi}$  is maximum measurement value,  $V_i$  is other measurement value and,  $i$  is different ranks in marketable yield.

**2.4. Data Analysis**

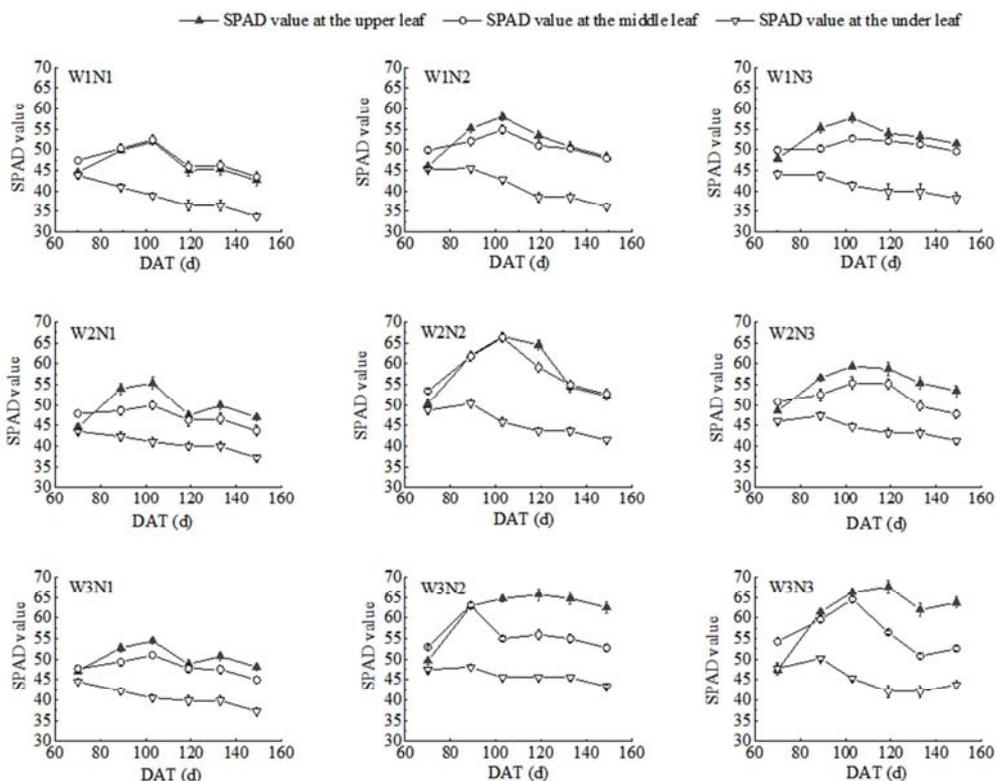
The data were analyzed by two-way analysis of covariance (ANCOVA) using DPS (Data Processing System). The significant difference of between mean values was separated multiple comparison tests at  $p \leq 0.05$  levels using LSD (Least-Significant Difference). Relationships between SPAD readings and other parameters were obtained by Linear or

Polynomial simulation, using Origin 2019.

**3. Results**

**3.1. Effects of Water and Nitrogen Treatments on SPAD Values and Nitrogen Accumulation**

The level of irrigation and nitrogen fertilizer application had a significant or extremely significant effect on SPAD values at different leaf positions. SPAD value at the middle of the leaf (MD-SPAD) and SPAD value of the upper leaf (UP-SPAD) was higher than SPAD value of under leaf (UD-SPAD), which gradually decreased under N1 by slightly increased first and then decreasing during growth period. MD-SPAD and UP-SPAD firstly increased and then decreased during growth period. However, UD-SPAD was significantly lower compared to other leaf positions. The SPAD values at different positions of N2 and N3 were both higher than those of N1. UP-SPAD and MD-SPAD at 103DAT recorded higher values of 66.28 and 66.25 under W2N2 treatment than under W2N3 and W3N3 treatments, respectively. There was a significant difference between W2N2 and W2N3 treatments as well as between W2N3 and W3N3 treatments. These values were much higher under W3N3 treatment than under W2N3 treatment (Figure 1). This indicates that application of nitrogen fertilizer should be appropriately increased with increasing level of irrigation to improve SPAD values. When the level of irrigation was W2, the optimum nitrogen fertilizer applied was N2.



**Figure 1.** Trend of SPAD values at different positions under different levels of irrigation and amount of nitrogen fertilizer applied during growth period, values are mean ± standard error. SPAD values adopted by 70DAT, 89DAT, 103DAT, 119DAT, 133DAT and 149DAT, respectively.

According to the values of nitrogen accumulation obtained at 150DAT (Table 3), the effects of level of irrigation and amount of nitrogen fertilizer applied on leaf nitrogen accumulation (LNA), fruit nitrogen accumulation (FNA) and total nitrogen accumulation (TNA) were significant. The nitrogen accumulation of root, stem, leaf and fruit ranged from 4.07-6.55kg·hm<sup>-2</sup>, 27.52-54.14kg·hm<sup>-2</sup>, 41.89-60.46kg·hm<sup>-2</sup>

and 80.81-157.46 kg·hm<sup>-2</sup> and representing nitrogen content of TNA of about 2%-4%, 16%-23%, 20%-27% and 49%-59%, respectively. This indicates that a half or more of TNA was found in the fruit, while the root had the least content. FNA and TNA were highest under W2N2 and W3N2 treatments, while N2 was the optimum nitrogen applied (Table 3).

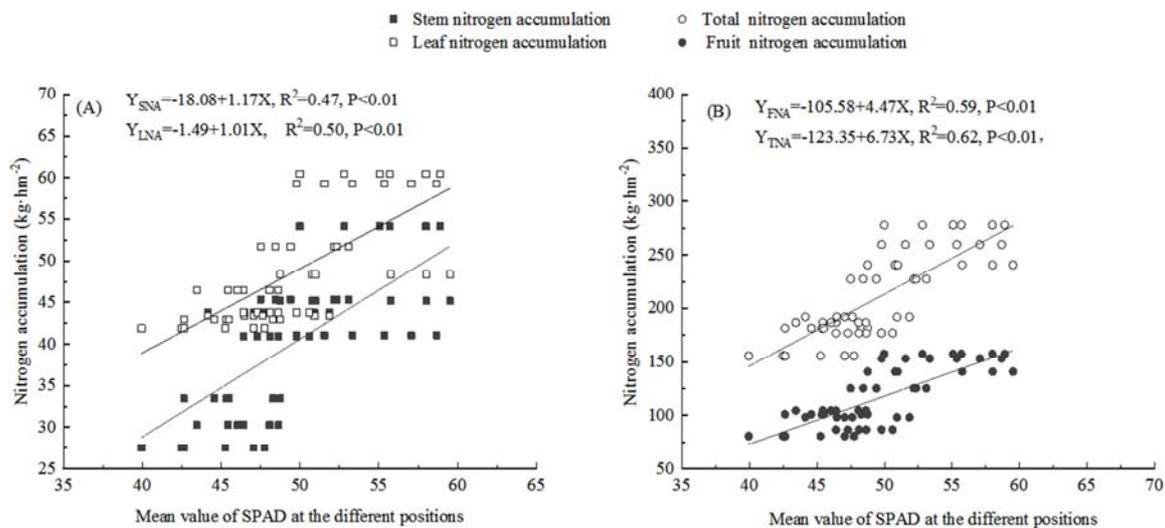
**Table 3.** Effects of irrigation level and nitrogen fertilizer treatments on nitrogen accumulation.

Treatments	RNA (kg·hm <sup>-2</sup> )	SNA (kg·hm <sup>-2</sup> )	LNA (kg·hm <sup>-2</sup> )	FNA (kg·hm <sup>-2</sup> )	TNA (kg·hm <sup>-2</sup> )
W1N1	5.43ab	27.52e	41.89d	80.81d	155.65d
W1N2	6.34a	43.77abc	43.39cd	98.37cd	191.88bcd
W1N3	5.25ab	40.91bcd	43.75cd	86.88d	176.81cd
W2N1	4.07b	33.46cde	42.95d	101.03cd	181.51cd
W2N2	6.55a	45.16ab	48.34cd	140.66ab	240.71ab
W2N3	5.05ab	45.31ab	51.70bc	125.20bc	227.27abc
W3N1	5.41ab	30.30de	46.46cd	104.52cd	186.69cd
W3N2	5.79ab	54.14a	60.46a	157.46a	277.86a
W3N3	6.35a	41.04bcd	59.25ab	153.10ab	259.74a
W	ns	ns	**	**	**
N	ns	**	**	**	**
W*N	ns	ns	ns	ns	ns

Notes: RNA, SNA, LNA, FNA, TNA represent nitrogen accumulation of root, stem, leaf, fruit and total nitrogen accumulation, respectively; \* represent F-test statistical significance at 0.05 probability level; \*\* represent F-test statistical significance at the 0.01 probability level; W, N and W\*N represent significant level of ANOVA of water stress effect, nitrogen fertilizer and the cross effect of two factors, respectively.

### 3.2. Relationships Between Nitrogen Accumulation and SPAD Values

SPAD values and nitrogen accumulation (SNA, LNA, TNA and FNA) were positively correlated, while the determinant coefficients (R<sup>2</sup>, n=54) ranged from 0.47-0.62 and P<0.01 (Figure 2). This indicates that SPAD values can be used for diagnosis of nitrogen uptake.



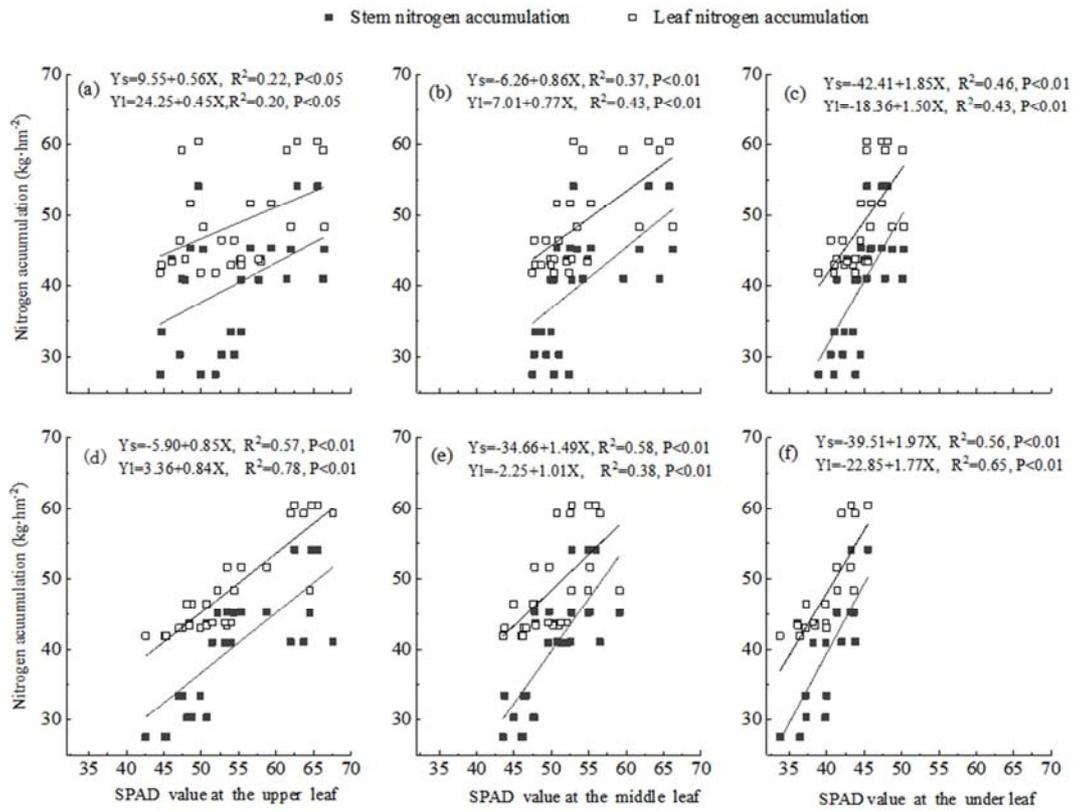
**Figure 2.** (A) Presents the relationships of SNA and LNA with mean value of SPAD values at different positions; (B) represent relationships of FNA and TNA with mean SPAD value at the different positions. SNA, LNA, FNA and TNA represent stem nitrogen accumulation and leaf nitrogen accumulation, fruit nitrogen accumulation and total nitrogen accumulation at 150DAT, respectively. Linear equations were shown, including determinant coefficients (R<sup>2</sup>) and P values.

The SPAD value at different positions were positively correlated with SNA and with LNA, with p<0.05 or p<0.01. The determinant coefficients of the relationships of UP-SPAD with SNA and with LNA, were lower (R<sup>2</sup>=0.22 and R<sup>2</sup>=0.20) than those of fruit expanding period (Figure 3a, b and c). This indicates that estimation of nitrogen content (SNA and LNA) was better via MD-SPAD and UD-SPAD during fruit

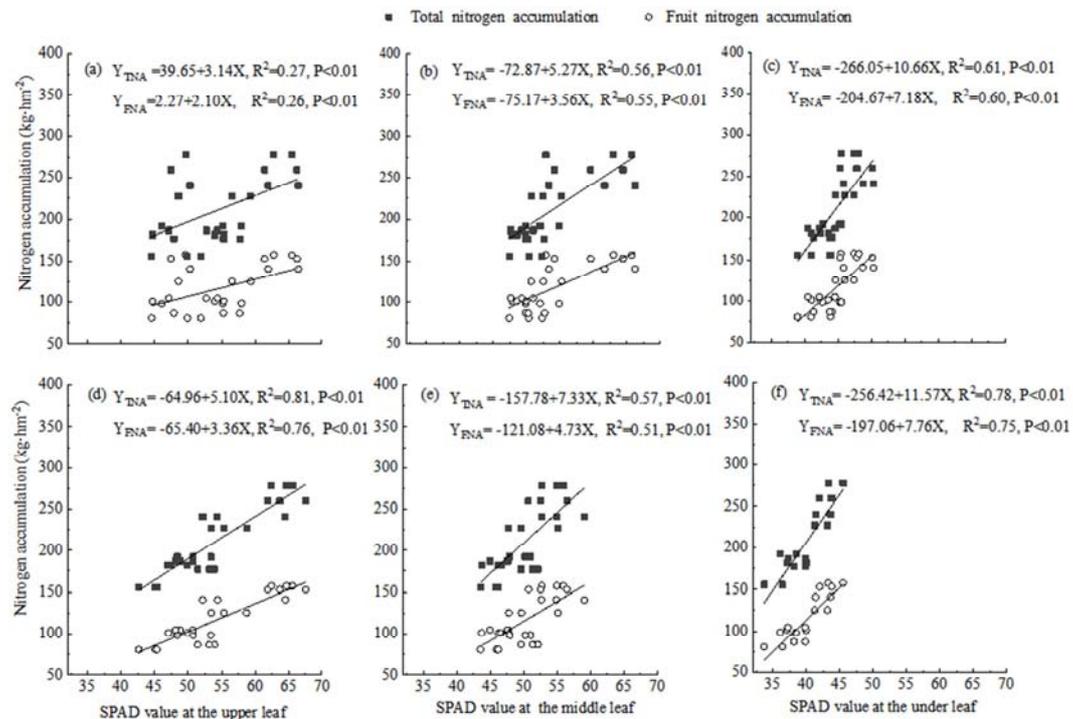
expanding period.

Without considering different leaf positions, the determinant coefficients were higher during mature period than during fruit expanding period except for the determinant coefficient of relationship between MD-SPAD and LNA (Figure 3d, e and f). This indicates that nitrogen accumulation (SNA and LNA) could be estimated using different leaf positions during mature

period, specifically, UP-SPAD was the best for diagnosis of SNA and LNA during growth.



**Figure 3.** (a),(b) and (c) represent relationships of SNA and LNA with SPAD value at the upper leaf (UP-SPAD), with SPAD value at the middle leaf (MD-SPAD) and with SPAD value at the under leaf (UD-SPAD) during fruit expanding period, respectively. (d),(e) and (f) represent relationships of SNA and LNA with UP-SPAD, with MD-SPAD and with UD-SPAD during mature period, respectively. Linear equations are shown, including determinant coefficients ( $R^2$ ) and  $P$  values.



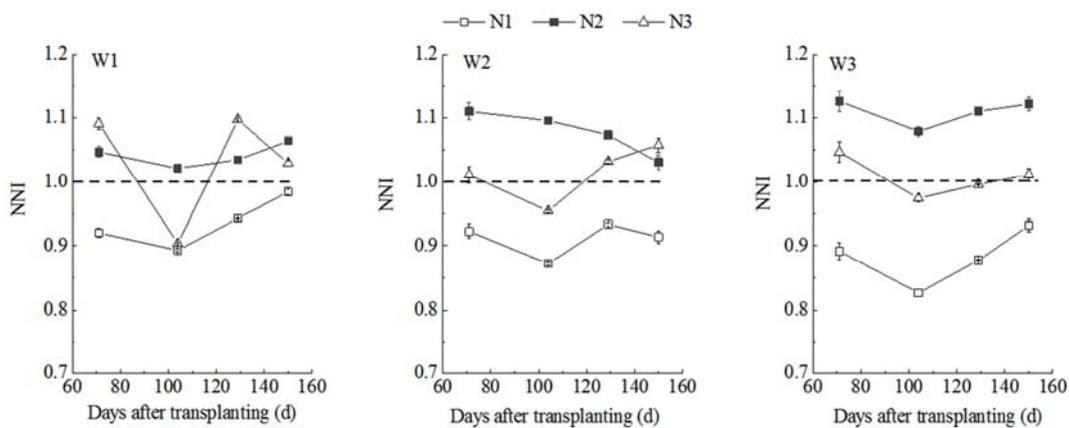
**Figure 4.** (a),(b) and (c) represent relationships of TNA and FNA with SPAD value at upper leaf (UP-SPAD), with SPAD value at middle leaf (MD-SPAD) and with SPAD value at under leaf (UD-SPAD) during fruit expanding period, respectively. (d),(e), and (f) represent relationships of TNA and FNA with UP-SPAD, with MD-SPAD, and with UD-SPAD during mature period, respectively. Linear equations are shown, including determinant coefficients ( $R^2$ ) and  $P$  values.

The SPAD values at different positions were positively correlated with TNA and FNA and were significantly different ( $p < 0.01$ ). The determinant coefficients of relationships between UP-SPAD and SNA and between UP-SPAD and LNA, were lower ( $R^2 = 0.22$  and  $R^2 = 0.20$ ) than the others, which were  $R^2 > 0.50$  (Figure 4). This indicates that estimation of nitrogen content (TNA and FNA) was better via MD-SPAD and UD-SPAD during fruit expanding fruit period, whereas UP-SPAD was the best indicator for diagnosis of TNA and FNA during mature period.

**3.3. Relationships Between Nitrogen Nutrition Index (NNI) and SPAD Values**

During growth period, the nitrogen nutrition index (NNI)

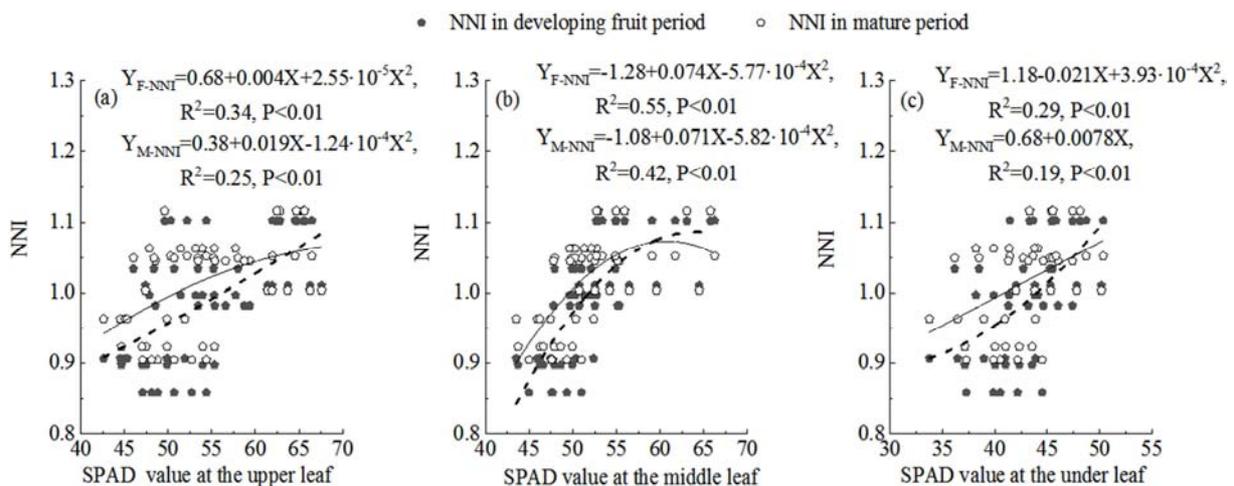
values were consistently less than those under low nitrogen treatment (N1), but higher than those under middle nitrogen treatment (N2). Different levels of irrigation had significant effects on NNI values, these had marked variation under high nitrogen treatment (N3) under different levels of irrigation. NNI values of N3 were slightly higher or lower than those of growth period under full irrigation (W3). This indicates that NNI values were optimum under W3N3 treatment, whereas NNI values of N2 were gradually close to those of mature period under mild water stress (W2), NNI values was also gradually close to one in that period under N1. Different nitrogen application rates were selected based on the degree of water stress, NNI values were better under W1N1 treatment and W2N2 treatment during mature period (Figure 5).



**Figure 5.** The dynamics of nitrogen nutrition index (NNI) values under different levels of irrigation and application of nitrogen fertilizer. Data of nitrogen concentration adopted at 71DAT, 104DAT, 129DAT, and 150 DAT.

There was a highly significant difference ( $p < 0.01$ ) between NNI and SPAD values in the expanding fruit period (F-NNI) and NNI in the mature period (M-NNI) at the different positions. The determinant coefficients of the relationships of

F-NNI and M-NNI with MD-SPAD were higher than others at  $R^2 < 0.35$  (Figure 6). This indicates that MD-SPAD has a better accuracy in diagnosing F-NNI and M-NNI.



**Figure 6.** (a), (b), and (c) are the relationships of NNI in fruit expanding period and NNI in mature period with SPAD value at the upper leaf (UP-SPAD), with SPAD value at the middle leaf (MD-SPAD) and with PAD value under leaf (UD-SPAD), respectively. Their regression equations are shown, including determination coefficients ( $R^2$ ) and P values; the dotted lines represent relationships between NNI in fruit expanding period with SPAD values at different positions; the continuous lines represent relationships between NNI in mature period with SPAD values at different positions. The NNI data in the fruit expanding period adopted mean of NNI at 71DAT and 104DAT, while the NNI data in the mature period adopted mean of NNI at 129DAT and 150 DAT.

### 3.4. Relationships Between Marketable Yield and SPAD Values

The marketable yield was divided into three levels based on single fruit in the local market, namely small fruit yield (SFY), middle fruit yield (MFY) and big fruit yield (BFY). These occupied the total marketable yield of 11%-19%, 52%-55% and 32%-36%, respectively. The total marketable yield mainly consisted of MFY and BFY, which initially increased and later

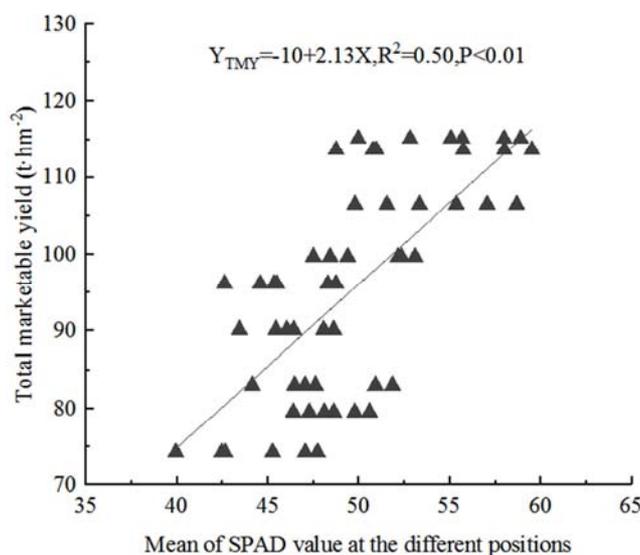
decreased with irrigation levels and nitrogen fertilizer increment. There were significantly different effects between irrigation levels and nitrogen fertilizer on MFY, BFY and TMY, which were higher under W2N2 and W3N2 treatments. Moreover, there were no significant difference between W2N2 and W3N2 treatments for MFY, BFY and TMY (Table 4). This indicates that the irrigation levels of W2 and nitrogen fertilizer of N2 are important in increasing MFY, BFY and TMY.

**Table 4.** Effects of irrigation levels and nitrogen fertilizer treatments on the marketable yield.

Treatments	SFY (F <sub>60-125</sub> ) (t·hm <sup>-2</sup> )	MFY (F <sub>125-250</sub> ) (t·hm <sup>-2</sup> )	BFY (F <sub>250-350</sub> ) (t·hm <sup>-2</sup> )	TMY (F <sub>60-350</sub> ) (t·hm <sup>-2</sup> )
W1N1	11.64cd	38.57e	23.99c	74.19e
W1N2	12.81bc	43.89de	26.27c	82.98de
W1N3	12.54bcd	41.04e	25.92c	79.50e
W2N1	12.32cd	51.02cd	30.75c	96.10bc
W2N2	12.66bc	59.81a	41.08a	113.56a
W2N3	13.58b	52.05bc	34.03b	99.66b
W3N1	12.32cd	48.47d	29.30c	90.10c
W3N2	14.54a	61.47a	39.04a	115.06a
W3N3	14.45ab	55.85ab	36.09ab	106.39ab
W	**	**	**	**
N	ns	**	**	**
W*N	ns	ns	*	**

Note: F<sub>60-125</sub>, F<sub>125-225</sub>, F<sub>250-350</sub> and F<sub>60-350</sub> represent single fruit weight (FW) ranging from 60g≤FW<125g, 125g≤FW<250g, 250g≤FW<350g and 60g≤FW<135g, respectively.

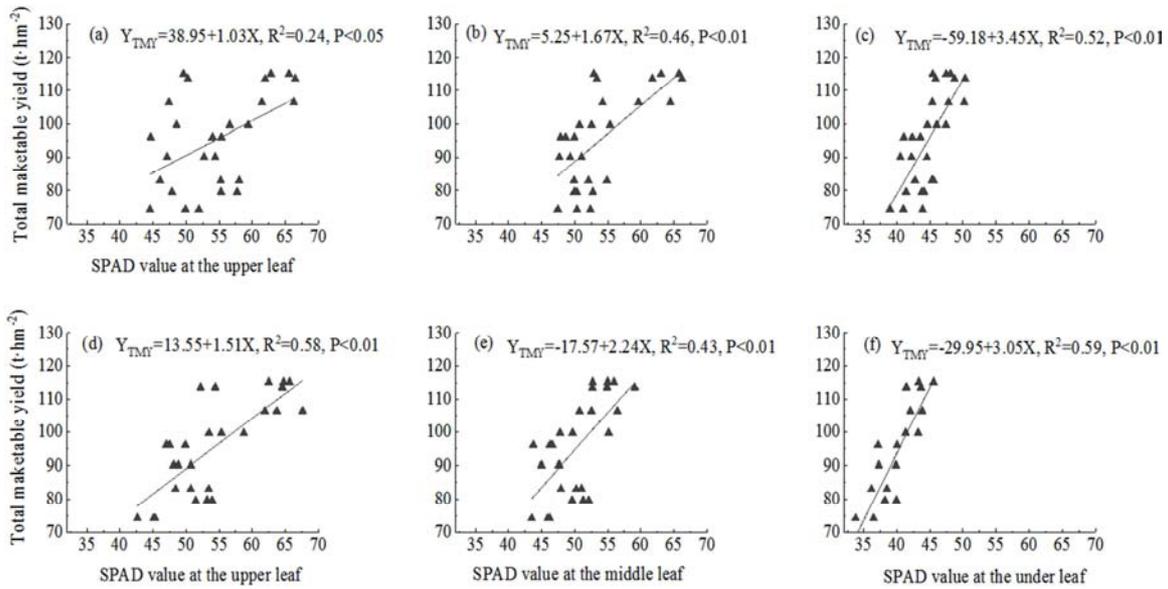
The mean SPAD value at the different positions was positively correlated with total marketable yield ( $R^2=0.50$ ,  $p<0.01$ ,  $n=54$ ) (Figure 7). This indicates that SPAD value are useful in analysis of total marketable yield.



**Figure 7.** The relationship between mean of SPAD values and total marketable yield at the different positions. The linear equation shown encompasses determination coefficient ( $R^2$ ) and  $P$  value. Data of mean of SPAD value at the different positions adopted at 70DAT, 89 DAT, 103 DAT, 119DAT, 133DAT and 149 DAT.

The TMY and SPAD values at the different positions were positively correlated and significantly different ( $p<0.05$  or  $P<0.01$ ). The determinant coefficient of the correlation between UP-SPAD and TMY was lower than others ( $R^2>0.42$ ). This indicates that estimating TMY was better through

MD-SPAD and UD-SPAD during the fruit expanding period. Moreover, UP-SPAD and UD-SPAD were better indicators of TMY during the mature period (Figure 8). The UD-SPAD was the best indicator for diagnosing TMY during the two growth periods.

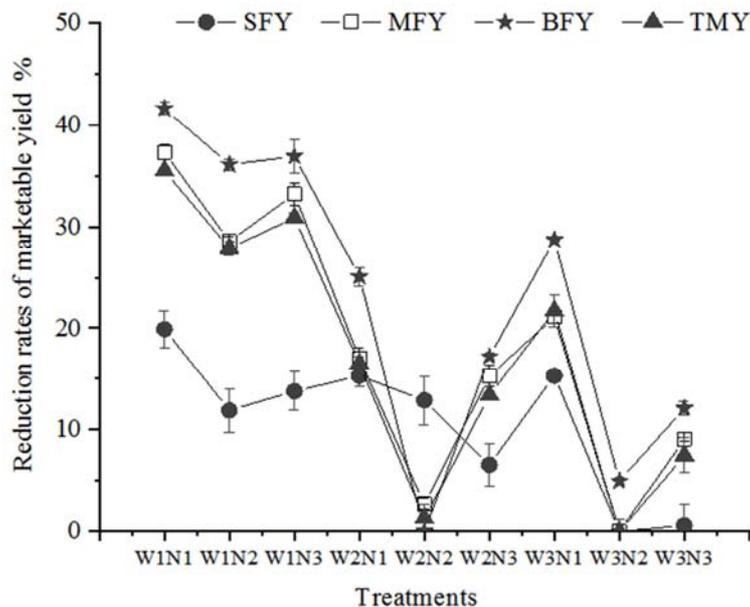


**Figure 8.** (a), (b) and (c) represent TNA relationships with SPAD value at the upper leaf (UP-SPAD), with SPAD value at the middle leaf (MD-SPAD) and with SPAD value at the under leaf (UD-SPAD) during fruit expanding period, respectively. (d), (e) and (f) represent TNA relationships with UP-SPAD, with MD-SPAD and with UD-SPAD during mature period, respectively. Respective linear equations are shown which encompasses determination coefficients (R<sup>2</sup>) and P values.

### 3.5. Quantification of Reduction Rates of the Marketable Yield

With worsening water stress, the reduction rates of different ranks in marketable yield (SFY, MFY, BFY) and total marketable yield (TMY) increased. Maximum values were

obtained under W1N1 treatment and minimum values at near zero were obtained under W2N2 treatment and W3N2 treatment. In the different treatments, the reduction rate of BFY was the highest, while reduction rate of SFY was the least (Figure 9). This indicates that BFY was significantly affected by water stress.

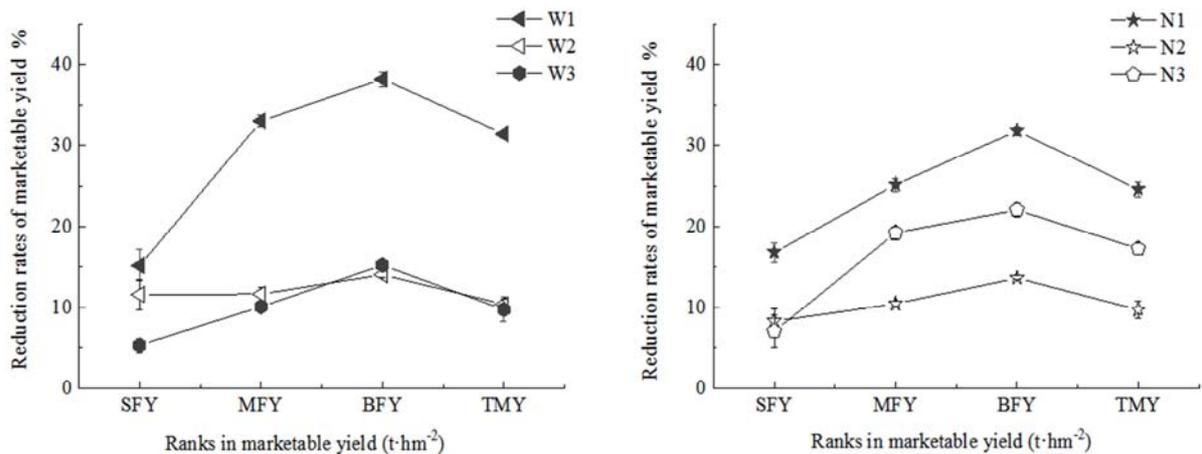


**Figure 9.** The reduction rates of marketable yield under the 9 different treatments. Values are mean±standard error.

The reduction rates of MFY, BFY, and TMY were highest under W1 and N1, ranging from 31%-38% and 25%-32%, respectively. In contrast, the reduction rates of MFY, BFY and TMY were not significantly reduced under W2. When the

nitrogen fertilizer application reached N3, the reduction rates of MFY, BFY and TMY increment was higher than N2 (Figure 10). This indicates that N1, N3 and W1 can highly reduce yield, especially at severe water stress of W1 in blossom and

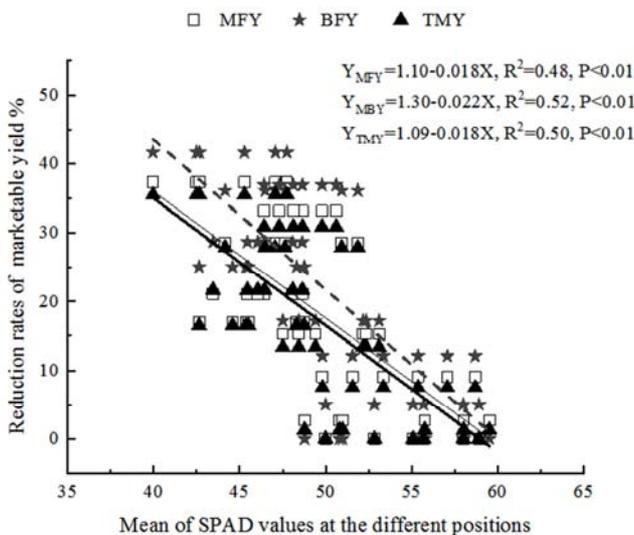
fruit period.



**Figure 10.** Quantification of reduction rates in marketable yield under three irrigation levels and three nitrogen fertilizer applications. Values are mean±standard error.

The reduction rates of marketable yield and mean of SPAD values at the different positions were negatively correlated and significantly different ( $R^2 > 0.47$ ,  $P < 0.01$ ,  $n = 54$ ) (Figure 10.). This indicates that SPAD values can be used to predict reduction rate in marketable yield, including MFY, BFY and TMY. The linear equations differed insignificantly (intercept, slope,  $R^2$ ) and  $p < 0.01$ .

(NNI), showed a strong relationship with SPAD value at different leaf positions. This result was consistent with previous studies which showed similar positive relationship of chlorophyll with nitrogen status in crops such as tomato [26] and cucumber [27]. The linear equations reached significant or extremely significant levels, whereas the determinant coefficients were different (Figure 3), where strong degree of relationship was different between SPAD values at different positions and nitrogen status. The weakest relationships were obtained between SPAD value at upper leaf (UP-SPAD) and nitrogen accumulation in fruit expanding period, but it was the strong relationship in mature period (Figure 3a and Figure 4a). SPAD values at upper leaf may be greatly impacted by re-watering during the mature period. Previous studies have shown that re-watering in milking stage can effectively improve chlorophyll content but it still declines in mature period [17, 28], indicating that declining trend of chlorophyll content may be mitigated to some extent in mature period by re-watering. However, the strongest relationships between NNI in fruit expanding period and SPAD value were obtained in the middle leaf (MD-SPAD), as well as between NNI in mature period and MD-SPAD, and all relationships were significant (Figure 6). These results agreed with those of previous studies that revealed that determinant coefficients were different and were most significant between SPAD indicators of different leaf positions and NNI in different growth periods [17].



**Figure 11.** The relationships of reduction rates of marketable yield and mean of SPAD value at the different positions. The linear equations are indicated and includes determination coefficients ( $R^2$ ) and  $P$  values.

## 4. Discussion

### 4.1. Estimating the Relationships of SPAD Values and Nitrogen Status

Nitrogen accumulation can be estimated via chlorophyll readings, by diagnosis of SPAD value at different leaf positions, but the accuracy of judgment may be different. Nitrogen status, including nitrogen accumulation of stem, leaf, fruit, total nitrogen accumulation and nitrogen nutrition index

The relationship between mean of SPAD values at different positions and nitrogen accumulation reached an extremely significant level in two the growth period. This indicated a good relationship between nitrogen accumulation and chlorophyll readings (Figure 2). Previous studies showed that the strongest relationship between chlorophyll readings and total-N content was realized when it was measured at the midpoint of the last fully developed leaf at the growth stage 49 [29]. However, dividing ratio of in growth period and leaf position, gives a more accurate relationship between nitrogen content and chlorophyll readings in the current study,

especially in estimation of NNI.

#### 4.2. Estimation of Relationships of SPAD Values with Marketable Yield

The total marketable yield had a positive relation with SPAD values at the different positions which reached extremely significant level (Figure 8), illustrating that the relationship of chlorophyll readings with total marketable yield was strong. The results were consistent with previous studies which revealed a positive relationship between chlorophyll readings and yield. In the current study, whereas, the relational degree was different in fruit expanding period, the strongest relationship was realized between UD-SPAD with total marketable yield (Figure 8c), followed by that between MD-SPAD and total marketable yield (Figure 8b). This indicates that the relationship was more accurate to be diagnosed by UD-SPAD and MD-SPAD, which may be greatly affected by full level of irrigation in seedling period. Earlier studies showed that the water deficit was affected significantly by total chlorophyll content which was also decreased in the vegetative stages [31], whereas chlorophyll readings at under leaf was lower than those of others, as it may not obtain full light because of being shaded by upper leaves in whole growth period. The results showed that UD-SPAD gradually declined or slightly increased first and then declined (Figure 1), due to lack of light. The study showed that the contents of chlorophyll a, chlorophyll b and chlorophyll (a + b) in leaves were reducing because of shading compared to full light [32].

The determinant coefficient ( $R^2=0.58$ ) of relationship of UP-SPAD with total marketable yield greatly improved in mature period (Figure 8d), due to availability of full light and re-watering, with gradual ageing of leaves, chlorophyll content was not improved by re-watering in mature period but chlorophyll readings of upper leaf was still high under W3N2 and W3N2 treatments (Figure 1). In the two growth stages, a strong relationship was obtained between mean of SPAD values at different positions and total marketable yield ( $R^2=50$ ,  $P<0.01$ ) (Figure 7), which was similar with a previous study which showed positive relationship between chlorophyll and yield ( $R^2=0.67$ ) [33].

#### 4.3. Effects of Water Stress and Application of Nitrogen Fertilizer on Marketable Yield

Ranks of marketable yield, including small fruit yield (SFY), middle fruit yield (MFY), big fruit yield (BFY), and total marketable yield (TMY) were higher under irrigation levels of W2 and W3 than under severe water stress (W1), W2 was the optimal level of irrigation for saving water without reducing production. This could be because severe water stress resulted in smaller fruit size than full irrigation or mild water stress. It was found that BFY was greatly decreased by W1 compared to W2 and W3 (Figure 10), this study was consistent with earlier findings that severe water stress resulted in smaller fruit size [34]. Application of Nitrogen fertilizer ( $300 \text{ kg}\cdot\text{hm}^{-2}$ ) was beneficial in increasing of MFY,

BFY, and TMY (Table 4 and Figure 10), which were restrained by application of excessive nitrogen fertilizer, previous researches showed that excessive nitrogen may cause soil degeneration, which led to nitrogen leaching and a decline of yield [3, 35]. Strong negative relationships were revealed between reduction rates of MFY, BFY, and TMY and mean of SPAD value at different positions (Figure 11), indicating a reducing rate in yield due to water stress and application of too much or less nitrogen fertilizer could be diagnosed through SPAD values.

It was found that TMY obtained an optimal value when the level of irrigation and applied nitrogen fertilizer were W2 ( $0.75W3$ ) and N2 ( $300 \text{ kg}\cdot\text{hm}^{-2}$ ), respectively (Table 4). However, previous studies showed that irrigation and fertilizer regime of  $0.75 \text{ Ep}$  (Ep is the cumulative evaporation from a standard 20-cm pan (mm)) and  $250 \text{ kg N ha}^{-1}$  were the best strategy of water and N management for production of drip-irrigated greenhouse tomatoes [36]. Difference could be due to different breed and irrigation plan, previous research showed that the screening of a large collection of tomato, including different fruit and cultivar types, revealed the existence of a high variability in yield [37] and different irrigation plans resulted in significantly difference in water saving and yield [38]. Re-watering in mature period, did not promote compensatory benefit of production, indicating that severe water stress in the critical period had impacted production hugely. Studies have shown that the severe water deficit (one-third of full irrigation) at the flowering and fruit development stage, result in decreased yield [39, 40].

## 5. Conclusions

Without considering growth period and the leaf positions individually, this study showed that SPAD values can be used to estimate nitrogen status, total marketable yield and reduction rates of marketable yield. When considering the growth period and leaf positions individually, SPAD value at middle leaf and under leaf may be more suitable for estimating nitrogen accumulation and total marketable yield in growth period, whereas SPAD value at upper leaf may be the most accurate diagnosis for nitrogen accumulation and total marketable yield in fruit expanding period which is opposite in mature period. Without considering growth period individually, SPAD value at the middle leaf could be a more accurate indicator of nitrogen nutrition index (NNI). When simultaneously considering estimation of nitrogen accumulation, NNI and marketable yield using chlorophyll readings, mean of SPAD values adopted by different leaf positions or SPAD value at the middle leaf could be used to more accurate estimate nitrogen content and yield.

SPAD values and nitrogen accumulation, including those of stem, leaf, fruit and total nitrogen accumulation, were higher under W2N2, W2N3, W3N2 and W3N3 treatments. Total marketable yield was optimal under W2 of  $2488 \text{ m}^3\cdot\text{hm}^{-2}$  and N2 of  $300 \text{ kg}\cdot\text{hm}^{-2}$  and reduction rate of big fruit yield was the highest than that of others, thus big fruit yield could be greatly affected by severe water stress in blossom and fruit periods.

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