

## Cell Membrane Stability and Leaf Water Relations as Affected by Nitrogen Application in Maize (*Zea mays* L.)

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**Abstract :** A field experiment was conducted to investigate the effect of N application on cell membrane stability measured by the polyethylene glycol test and leaf water relations in maize. With increasing N levels leaf water potential was decreased while total plant weight and cell membrane stability were increased. Osmotic potential decreased with increasing N levels in order to maintain turgor at lower water potential conditions. Sugar and K were identified as the major osmotic contributors in maize. Osmotic adjustment was evident while concentrations of sugar, K and amino acids in cell sap increased with increasing N application.

**Key Words :** Cell membrane stability, Leaf water potential, Nitrogen nutrition, Osmotic adjustment, *Zea mays* L.

圃場に生育するトウモロコシ葉の細胞膜安定性および葉の水分状態に及ぼす窒素施与の影響：ニャーナシリ S. プレマチャンドラ・実岡寛文・松浦秀明・尾形昭逸（広島大学生物生産学部）

**要 旨：**窒素施与量を 150, 250 および 350 kg/ha（それぞれ少，中，多窒素区とする。）の 3 段階に変えた圃場条件下でトウモロコシ（品種 P 3358）を栽培し，播種後 70 日目に展開完了最上位葉についてポリエチレングリコール法による葉の細胞膜安定性，葉の水ポテンシャル，浸透ポテンシャルおよび細胞液中の溶質濃度を測定し，葉の水分状態と細胞膜安定性に及ぼす窒素施与の影響を明らかにした。

地上部新鮮重は窒素施与に伴い増加し，少窒素区に対する多窒素区のその増加率は約 4.1 倍であった。3 処理区の葉の水ポテンシャルは少>中>多窒素区の順に低く，多窒素区ほど葉の水ポテンシャルの低下は大きかった。しかし，水ストレスに対する葉の細胞膜安定性は，逆に多窒素区ほど高かった。多窒素区の葉の浸透ポテンシャルは，中および少窒素区に比べて低かったが，膨圧は逆に少窒素区に比べて多窒素区で著しく高かった。窒素施与量の増加にともない葉細胞液中のカリ，カルシウム，リン，窒素，アルコール可溶糖さらには遊離アミノ酸濃度が著しく増加した。

以上の結果，多窒素区では少窒素区に比べて葉の水ポテンシャルが低下したにもかかわらず，地上部新鮮重および細胞膜安定性の増加が認められ，その主たる要因として糖，カリ，窒素の集積による葉の浸透調整力の増大にともない，葉の膨圧が高く維持されるためと考えられる。

**キーワード：**細胞膜安定性，浸透調整，窒素栄養，トウモロコシ (*Zea mays* L.)，葉の水ポテンシャル。

Evaluation of cellular membrane integrity seems to be efficient as a measure of drought and heat tolerance. The method for measuring drought tolerance using polyethylene glycol (PEG) introduced by Sullivan<sup>14,15)</sup> was found to be comparatively efficient in estimating drought tolerance of wheat (*Triticum aestivum* L.)<sup>8)</sup>, orchardgrass (*Dactylis glomerata* L.)<sup>7)</sup> and maize (*Zea mays* L.)<sup>9)</sup>.

Physiological responses of plants to water stress and N nutrition have been extensively investigated. Nitrogen nutrition and water stress interact to control several processes in plants. Nitrogen deficiency sensitized the plants to water stress and the effects of stress

occurred at a higher water potential<sup>11)</sup>. Morgan<sup>6)</sup> indicated that the interaction of water stress and N nutrition on plant response have received relatively little attention and he encouraged studies on N nutritional effects on osmotic adjustment under conditions of comparable water stress regimes. Adequate studies have not been conducted on interactive effects of N nutrition and water stress on substances active in osmotic concentration.

The purpose of the present study was to investigate the effect of nitrogen nutrition on cell membrane stability (CMS) measured by the PEG test, plant water relations and solute and mineral concentrations in leaf tissues in maize, under limited soil moisture conditions.

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## Materials and Methods

Maize cultivar, Pioneer 3358 (maturity 125 days) was grown in the experimental farm of Hiroshima University during June to September 1988. Three N levels (150, 250 and 350 kg/ha) were tested in the field plots of  $3.2 \times 4$  m on a randomized block design with three replications. Nitrogen was applied as ammonium sulphate. Soil was sandy loam with pH 5.6. The basal fertilizer application was made with 50, 100 and 150 kg/ha respectively of the three N levels with 200 kg/ha of K and 150 kg/ha of P. The balance of N fertilizer was top dressed when the plants were 30 days old. Planting was done after the monsoon rains with rows spaced 80 cm apart and 20 cm spacing between plants. Plants were not irrigated throughout the experiment and received a comparatively less rainfall (51.7 cm during the 110 days). Measurements except the total plant weight were done when the plants were 70 days old using the uppermost fully expanded leaves, with three replications.

### *Cell membrane stability measurement by the PEG test*

Discs of 1.1 cm diameter were cut from the middle portion of the leaves using a leaf punch. Thirty leaf discs were placed in a 100 ml flask and washed three times with deionized distilled water. For the desiccation treatment, leaf discs were then submerged in 30 ml of 30% PEG 600 solution (osmotic potential  $-2.48$  MPa) and allowed to stand in the solution for 24 h at  $10^\circ\text{C}$ . The leaf discs were then washed rapidly three times with deionized distilled water. Both desiccated and control samples were immersed in 30 ml of deionized distilled water for 24 h at  $10^\circ\text{C}$ . The flask was warmed to  $25^\circ\text{C}$  and shaken well, and the electrical conductivity was measured using the Electric Conductivity Meter (TOA CM-6A). Following the conductivity measurement, the leaf tissues were killed by autoclaving for 15 min and electrical conductivity was measured at  $25^\circ\text{C}$ . Three replicates were measured for the desiccation treatment (T) and non-desiccated control (C).

Cell membrane stability of leaf tissues was calculated as the percentage injury using the equation,  $\text{Percentage injury} = 1 - (1 - T_1/T_2) / (1 - C_1/C_2) \times 100$ , where  $T_1$  and  $T_2$  = first and second conductivity measurement of

the desiccation treatment, respectively,  $C_1$  and  $C_2$  = first and second conductivity measurement of control, respectively.

### *Measurement of leaf water potential and osmotic potential*

Leaf water potential and osmotic potential were measured using a thermocouple psychrometer (Wescor micro voltmeter and C-52 sample chamber). Discs of 0.5 cm diameter were cut from the middle portion of the leaves using a leaf punch at sometime between 10.00 and 13.00 h, immediately put into the sample chambers and were transported to the laboratory. Leaf water potential readings were taken by the psychrometric method after an equilibration period of 1 h. Thereafter the leaf discs were put into 0.6 ml plastic seal tubes and frozen (at  $-20^\circ\text{C}$ ). The frozen leaf discs were thawed and used for the measurement of osmotic potential by the psychrometric method after an equilibration period of 20 min.

### *Nutrient analysis in dry Matter and cell sap*

Mid portions of the leaves were sampled, frozen at  $-20^\circ\text{C}$ , thawed and centrifuged at  $1000 \times g$  to extract cell sap. Leaf tissues were freeze-dried and digested to prepare sample solutions. Cell sap and dry matter were analysed for sugar, Na, K, Ca, Mg, P and N concentrations. Sugar (80% hot ethanol soluble) was determined by the anthrone method<sup>16</sup>. Sodium and K were measured using a flame photometer (Eiko Pla). Magnesium and Ca were measured using an atomic absorption spectrophotometer (Hitachi 208). Total P was determined by the molybdenum blue method<sup>16</sup>. Total N in cell sap and dry leaf tissues was determined by Kjeldahl digestion followed by steam distillation. Concentration of amino acids in cell sap was determined using a Hitachi 835 Amino Acid Analyzer. Sucrose, glucose and fructose concentrations in cell sap were measured by enzymatic analysis, UV-method<sup>11</sup>. The enzymes were obtained from the Boehringer Mannheim Corporation (London) Ltd (Lewes, Sussex, U.K.).

### *Measurement of total plant weight*

The total plant fresh weight and dry weight were measured when the plants were 110 days old.

## Results and Discussion

Percentage injury in the PEG test, leaf

water potential, osmotic potential, turgor potential and total plant weight at three N levels are shown in Table 1. Percentage injury decreased with increasing N levels and the differences between the N levels were statistically significant. The lower injury of leaf tissues indicate the higher CMS under higher N levels.

Leaf water potential decreased significantly with increasing N levels. Decrease in leaf water potential suggests that the plants have been stressed under higher N levels. Decrease in leaf water potential under higher N levels have been observed in various crop plants<sup>6,10,11</sup>. Radin *et al.*<sup>12</sup> observed decrease in leaf water potential with higher N supplies by about 0.4 MPa in cotton (*Gossypium hirsutum* L.) and they indicated that the decrease was for stomatal closure. In the present study leaf water potential decreased by 0.42 MPa at the highest N level compared to the lowest N level.

With higher supplies of N fertilizer, plant production increases rapidly<sup>2</sup>. In the present study the total plant fresh weight increased by 38% at the highest N level compared to the lowest N level. Nitrogen and water influence dry matter production mainly by increasing leaf area duration and net assimilation rate<sup>5</sup>. Since the plants absorb much water from the soil for increased production, deficits in soil moisture may occur at higher N levels compared to lower N levels under limited soil moisture conditions, resulting decreases in leaf water potential.

Osmotic potential decreased with increasing N levels in order to maintain turgor at lower water status, which in turn enables plants to remain processes such as cell enlargement and stomatal opening. The results indicated substantial adaptation to water deficits via osmotic adjustment and turgor maintenance.

Osmotic adjustment of 1.01 MPa was evident at the highest N level compared to the lowest N level. Morgan<sup>6</sup> observed an osmotic adjustment of 0.29 MPa with higher N supplies under limited soil moisture in wheat. Turgor potential increased with increasing N levels in order to maintain the absorption of water at a lower leaf water potential.

Increased CMS with increasing N levels may be a result of the acclimation of plants to water stress. Saxon *et al.*<sup>13</sup> described the primary effect of drought on the membrane as a loss of turgor pressure, changing the mechanical stress on the membrane and its interaction with the cell wall. Premachandra *et al.*<sup>9</sup> observed decreases in percentage injury in the PEG test under low soil moisture conditions in maize. They found significant correlations of CMS with leaf water potential and osmotic potential and suggested that osmotic adjustment may have affected solute leakage.

Table 2 illustrates the concentration of Na, K, Ca, Mg, P, total N and sugars in cell sap at three N levels. Potassium, Ca, P, N and total sugar concentrations increased while Mg and Na decreased with increasing N levels. Glucose and fructose concentrations increased considerably while sucrose did not increase with increasing N levels. Table 3 illustrates the concentration of amino acids in cell sap at three N levels. Concentrations of all the amino acids detected except cystine increased with increasing N levels resulting a more than 100% increase in the concentration of total amino acids at the highest N level compared to the lowest N level. In the dry matter analysis, sugar, Na, K, Ca, Mg, P and N concentrations in leaf tissues increased with increasing N levels (Table 4). The differences between the lowest and the highest N levels were significant except for Mg. Sugar and K were

Table 1. Percentage injury in the PEG test, leaf water potential, osmotic potential, turgor potential and total plant weight in maize cultivar, Pioneer 3358 grown under three nitrogen levels.

Nitrogen level (kg/ha)	Percentage injury in PEG test	Leaf water potential (-MPa)	Osmotic potential (-MPa)	Turgor potential (MPa)	Total Plant weight (kg/m <sup>2</sup> )	
					fresh	dry
150	22.5±3.0	1.02±0.09	1.21±0.11	0.19±0.09	2.9±0.5	1.0±0.2
250	13.3±1.1	1.06±0.26	1.50±0.11	0.44±0.09	3.4±0.2	1.2±0.1
350	10.2±0.4	1.44±0.07	2.22±0.07	0.78±0.18	4.0±0.3	1.3±0.0

\* : Mean±standard error.

Table 2. Concentration of Na, K, Ca, Mg, P, total N and sugars in cell sap in maize cultivar, Pioneer 3358 grown under three nitrogen levels.

	Nitrogen level (kg/ha)		
	150	250	350
	Concentration in cell sap (mmol/l)		
Na	0.002±0*	0.001±0	0.001±0
K	175.0±3.7	183.0±6.1	191.3±6.9
Ca	14.4±2.2	15.8±2.9	20.1±1.7
Mg	4.9±0.9	4.1±0.4	3.1±0.4
P	8.2±0.0	9.1±0.5	12.0±0.6
Total N	57.1±6.0	70.7±2.5	77.9±0.1
Total sugars	111.4±3.5	126.3±0.5	128.1±4.6
Glucose	30.3±1.1	33.9±1.9	43.3±1.5
Sucrose	21.9±5.5	13.5±0.9	14.3±1.9
Fructose	20.5±0.5	23.4±2.8	28.1±2.9

\* : Mean±standard error.

Table 3. Concentration of amino acids in cell sap in maize cultivar, Pioneer 3358 grown under three nitrogen levels.

Amino acid	Nitrogen level (kg/ha)		
	150	250	350
	Concentration (mmol/l)		
Asparatic acid	0.12±0.01*	0.39±0.04	0.56±0.09
Threonine	0.20±0.01	0.38±0.03	0.56±0.04
Serine	0.86±0.04	1.70±0.13	2.05±0.09
Glutamic acid	0.26±0.05	0.39±0.08	0.69±0.01
Proline	N.D.	N.D.	N.D.
Glycine	N.D.	N.D.	N.D.
Alanine	3.11±0.20	4.98±0.24	7.81±0.62
Valine	N.D.	N.D.	0.23±0.01
Cystine	1.38±0.02	1.21±0.01	1.29±0.06
Methionine	0.40±0.03	0.48±0.02	0.55±0.03
Isoleucine	0.34±0.01	0.40±0.03	0.46±0.04
Leucine	N.D.	0.12±0.02	0.14±0.03
Tyrosine	N.D.	N.D.	0.10±0.02
Phenylalanine	N.D.	N.D.	N.D.
Lysine	0.06±0.01	0.23±0.02	0.29±0.02
Histidine	N.D.	N.D.	N.D.
Arginine	N.D.	N.D.	N.D.
Total	6.73	10.28	14.73

\* : Mean±standard error, N.D. : Not detected.

the primary osmotic substances and K contributed more than sugar (Table 2). Premachandra *et al.*<sup>9)</sup> observed sugar and K as the primary osmotic contributors in maize and they found a positive correlation of K concentration with CMS measured by the PEG test. While the primary osmoticum varies somewhat with crop, K and sugar are contributors to osmotic adjustment in many plant species<sup>3,4)</sup>. Radin and Parker<sup>10)</sup> observed decrease in sugar, organic acids and other

solubles and a little increase in amino acids with high levels of N nutrition in cotton. The increase in N concentration at higher N levels could be accounted to the increased concentration of amino compounds, specially, amino acids with increasing N nutrition, and they also contribute to the osmotic potential.

The results indicated adaptation to water deficits with increasing N nutrition by increasing CMS and osmotic adjustment. Radin and Parker<sup>10)</sup> indicated that the magnitude of

Table 4. Sugar, Na, K, Ca, Mg, P, and N concentrations in leaf tissues in maize cultivar, Pioneer 3358 grown under three nitrogen levels.

Nitrogen level (kg/ha)	Sugar	Na	K	Ca	Mg	P	N
Concentration in dry leaf tissue (mg/g)							
150	68.0±3.6*	0.05±0.01	22.0±1.0	2.07±0.09	0.74±0.04	2.96±0.35	21.5±2.2
250	83.2±3.5	0.06±0.02	24.3±0.2	2.21±0.45	0.75±0.12	3.83±0.24	29.1±1.4
350	85.4±1.1	0.08±0.02	25.4±0.6	2.54±0.04	0.77±0.03	5.19±0.73	32.1±1.5

\* : Mean±standard error.

osmotic adjustment during adaptation to water deficits is much greater than the small differences in leaf water potential brought about by N and they suggested that osmoregulation is independent of N status. The present study does not clarify whether the osmotic adjustment observed at higher N levels was independent of N status. Further studies are necessary to find out the relationship between N nutrition and osmoregulation.

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