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# PHOSPHOLIPID CHANGES IN WHEAT AND BARLEY LEAVES UNDER WATER STRESS

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**Key Word Index**—*Triticum aestivum*; wheat; *Hordeum vulgare*; barley; Gramineae; phospholipids; tillering; ear emergence; grain filling; water stress.

**Abstract**—Total phospholipid content of leaves of wheat and barley increased and phospholipid components changed under water stress. Notable among these were the absence of phosphatidyl serine in barley varieties, decrease in phosphatidyl glycerol content in a less drought-tolerant variety of wheat (S-308) and barley (BG-25), and appearance of phosphatidic acid in both crops. The phospholipid content and its components did not return to normal upon release of the stress by subsequent irrigations. Such observations are indicative of water stress effected alterations in membranes.

## INTRODUCTION

Drought conditions severely limit crop production and in an attempt to alleviate this problem, several studies have been conducted [1]. Most of these concern carbohydrate [2], protein [3] and nucleic acids [4]. However, it appears that the effect of drought and simulated water stress on phospholipids (PL), which are important constituents of membranes [5] has not been studied. Water stress leads to the destruction of membrane structure [6]. The maintenance of membrane structure is of primary importance to ensure autonomy and proper functioning of various subcellular organelles. Water stress, while affecting membranes, would also lead to changes in phospholipids (PL) and the extent of such changes might indicate the drought tolerance of plants. Therefore, the effect of water stress was studied on the PL of leaves of two crops, wheat and barley having different water requirements. Two varieties each of wheat, S-308 (less drought tolerant) and C-306 (more drought tolerant), and barley, BG-25 (less drought tolerant) and C-138 (more drought tolerant), were selected.

## RESULTS

Total PL content of barley leaves at different stages was very similar to that of wheat (Tables 1 and 2). However, cv C-138 of barley had a higher PL content at all the stages. There was a marked reduction in PL content at the grain filling stage. Five major PLs viz. phosphatidyl choline (PC), phosphatidyl glycerol (PG), phosphatidyl serine (PS), phosphatidyl inositol (PI) and phosphatidic acid (PA) were present in leaves. Of these, PS was absent, while PI was more abundant in barley varieties. In both crops, the PC content showed a decrease and PA was absent from the ear emergence stage onwards.

Water stress caused an increase in the total PL content of leaves of both crops. The increase was more in the C-306 variety of wheat and the C-138 variety of barley. The maximum increase was *ca* 30% observed under stress in C-138 leaves at the tillering stage. Of the different PL components, PC and PI increased, while PA, which was absent in unstressed leaves, appeared under stress in both crops. The increase in PC content was more in S-308 and BG-25 varieties of wheat and barley, respectively, and it was maximum at the tillering stage. PG content decreased under stress only in less drought resistant varieties of wheat and barley, but the PS content decreased under stress in wheat, while it was absent in both barley varieties.

Changes in total PL content and its components were observed upon release of stress by subsequent rewatering of plants (Table 3). The total PL, PC and PI content remained higher and PA did not disappear in stress recovered plants. However, PS remained absent in barley varieties, while it was near normal in wheat plants. A low PG content in both crops was also observed in stress-released plants as compared to unstressed plants. These changes in different components were more normal in C-306 and C-138 varieties of wheat and barley respectively.

## DISCUSSION

The PL content of a cell has been considered to be an approximate measure of its membrane content [7]. Any change in PL content would suggest the synthesis or degradation of membranes. Thus, an increase in PL content under water stress would indicate an increase in the membrane content and this may be an adaptation mechanism to prevent damage due to drought. Similar PL increases have been observed as an adaptation to salt stress in cotton [8], barley and wheat [9].

Table 1. Effect of water stress on phospholipid composition of wheat leaves (mg/g dry wt)

| | Tillering | | Ear emergence | | Grain filling | |
|---|---|---|---|---|---|---|
| | C | S | C | S | C | S |
| Cv S-308 | | | | | | |
| Total phospholipid | 17.39±0.10 | 19.32±0.15 | 16.53±0.06 | 18.23 0.05 | 10.66±0.11 | 11.83±0.11 |
| PC | 6.62±0.15 | 11.20±0.09 | 5.76±0.08 | 8.65±0.07 | 3.20±0.18 | 4.47±0.13 |
| PG | 4.52±0.09 | 3.86±0.06 | 4.96±0.04 | 4.38±0.06 | 4.60±0.21 | 3.93±0.22 |
| PI | 1.97±0.10 | 2.30±0.11 | 2.15±0.06 | 3.44±0.10 | 1.69±0.11 | 2.71±0.18 |
| PS | 0.17±0.01 | 0.15±0.02 | 0.38±0.02 | 0.23±0.04 | 0.32±0.05 | 0.29±0.01 |
| PA | 0.09±0.01 | 0.37±0.02 | nil | 0.64±0.01 | nil | 0.23±0.02 |
| Cv C-306 | | | | | | |
| Total phospholipid | 16.84±0.15 | 20.69±0.08 | 16.94±0.14 | 19.32±0.09 | 13.60±0.19 | 16.70±0.15 |
| PC | 7.58±0.11 | 11.23±0.05 | 6.98±0.12 | 9.85±0.07 | 3.96±0.12 | 5.18±0.10 |
| PG | 4.04±0.10 | 4.14±0.08 | 5.59±0.20 | 5.75±0.16 | 4.86±0.18 | 4.80±0.10 |
| PI | 1.65±0.09 | 1.90±0.11 | 1.54±0.07 | 2.32±0.05 | 1.51±0.08 | 2.25±0.05 |
| PS | 0.22±0.05 | 0.20±0.01 | 0.45±0.01 | 0.40±0.06 | 0.40±0.01 | 0.38±0.03 |
| PA | 0.05±0.005 | 0.27±0.04 | nil | 0.39±0.01 | nil | 0.30±0.04 |

The results are mean ± s.d. of 4 replicate analyses. C and S refer to control and stress, respectively.

Table 2. Effect of water stress on phospholipid composition of barley leaves (mg/g dry wt)

| | Tillering | | Ear emergence | | Grain filling | |
|---|---|---|---|---|---|---|
| | C | S | C | S | C | S |
| Cv BG-25 | | | | | | |
| Total phospholipid | 16.68±0.15 | 19.00±0.08 | 16.00±0.11 | 17.50±0.09 | 12.94±0.05 | 14.69±0.11 |
| PC | 5.73±0.09 | 11.02±0.06 | 5.00±0.07 | 6.75±0.13 | 4.47±0.16 | 6.03±0.14 |
| PG | 3.84±0.08 | 3.43±0.08 | 4.14±0.05 | 3.72±0.13 | 4.03±0.18 | 3.62±0.10 |
| PI | 2.34±0.12 | 3.17±0.10 | 2.77±0.09 | 3.15±0.06 | 2.32±0.11 | 2.62±0.10 |
| PS | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| PA | 0.10±0.01 | 0.38±0.02 | nil | 0.67±0.05 | nil | 0.41±0.02 |
| Cv C-138 | | | | | | |
| Total phospholipid | 18.25±0.07 | 23.79±0.19 | 19.13±0.05 | 23.91±0.31 | 15.02±0.17 | 19.31±0.16 |
| PC | 9.18±0.10 | 12.31±0.08 | 8.45±0.07 | 10.38±0.06 | 4.80±0.12 | 5.54±0.10 |
| PG | 3.94±0.15 | 4.02±0.11 | 5.01±0.05 | 5.05±0.10 | 4.65±0.06 | 4.62±0.13 |
| PI | 2.18±0.05 | 2.50±0.09 | 3.06±0.11 | 3.98±0.06 | 2.78±0.15 | 3.70±0.10 |
| PS | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| PA | 0.08±0.01 | 0.22±0.03 | nil | 0.32±0.02 | nil | 0.26±0.03 |

The results are mean ± s.d. of 4 replicate analyses. C and S refer to control and stress, respectively. N.D. = not detectable.

Table 3. Phospholipids of wheat and barley leaves after release of stress (mg/g dry wt)

| | Wheat | | | | Barley | | | |
|---|---|---|---|---|---|---|---|---|
| | Cv S-308 | | Cv C-306 | | Cv BG-25 | | Cv C-138 | |
| | $S_1$ | $S_2$ | $S_1$ | $S_2$ | $S_1$ | $S_2$ | $S_1$ | $S_2$ |
| Total phospholipid | 11.72±0.09 | 12.60±0.16 | 15.30±0.12 | 14.96±0.18 | 14.10±0.08 | 13.97±0.08 | 18.61±0.13 | 17.71±0.05 |
| PC | 4.32±0.17 | 4.29±0.09 | 4.98±0.08 | 4.36±0.01 | 5.45±0.06 | 5.20±0.08 | 5.26±0.15 | 4.86±0.25 |
| PG | 4.05±0.38 | 4.16±0.09 | 4.82±0.13 | 4.82±0.17 | 3.67±0.08 | 3.91±0.40 | 4.60±0.14 | 4.46±0.18 |
| PI | 2.69±0.13 | 2.71±0.12 | 2.30±0.14 | 2.27±0.11 | 2.63±0.14 | 2.69±0.08 | 3.72±0.05 | 3.61±0.15 |
| PS | 0.29±0.02 | 0.29±0.01 | 0.39±0.01 | 0.39±0.04 | N.D. | N.D. | N.D. | N.D. |
| PA | 0.30±0.02 | 0.62±0.06 | 0.34±0.01 | 0.35±0.01 | 0.49±0.04 | 0.52±0.02 | 0.27±0.03 | 0.29±0.01 |

The results are mean ± s.d. of 4 replicate analyses. $S_1$ and $S_2$ refer to release of stress after tillering and ear emergence, respectively. N.D. = not detectable.

Under water stress, reduction in PG content was observed only in less drought tolerant varieties of both crops. The decrease in PG content may result in reduced photosynthesis since the glycerol of PG serves as a reservoir of phosphoglycerate [10] which is involved in $CO_2$ assimilation and PG also contains *trans*-3-hexadecenoic acid which is essential for the activity of photosystem II of photosynthesis [11, 12].

In both crops, PC content increased under stress, the increase being more prominent in wheat as compared to barley. Increase in PC content is known under adverse climatic conditions such as temperature [13] and salinity [4]. Leaf PI also increased under stress. Since both PI and PC are components of non-photosynthetic membranes like mitochondria, golgi apparatus and endoplasmic reticulum [15–17] it suggests that under water stress the proportion of non-photosynthetic membranes increases. The increase in PA content under water stress could be due to enhanced combined hydrolytic action of various lipid hydrolysing enzymes [18]. There was a little increase in PS content of wheat leaves under water stress, while it was absent in both varieties of barley. The absence of PS in barley, which requires much less water as compared to wheat, has also been reported by Kates [5]; however, its significance is not known. The release of stress, in general has positive effect on PL in both crops. This indicates that the effect of water stress was reversible and the extent of reversibility was more in drought resistant varieties of wheat (C-306) and barley (C-138).

## EXPERIMENTAL

Wheat varieties S-308 and C-306 and barley varieties BG-25 and C-138 were obtained from the Plant Breeding Department of this University.

*Cultivation under water stress.* Crops were sown in the field in *rabi* (winter) season. $H_2O$ stress was created by withholding irrigation at tillering, ear emergence and grain filling stages. The creation of stress was indicated by a decrease in the leaf $H_2O$ potential of stressed plants as indicated by a thermocouple psychrometer [9]. Release of $H_2O$ stress of plants stressed at tillering and ear emergence was done by giving normal irrigation subsequently.

*Lipid extraction and purification.* Leaves (10 g) were first boiled in 150 ml *iso*-PrOH for 10 min before homogenisation to denature lipolytic enzymes and then blended with 190 ml of $CHCl_3$—MeOH—$H_2O$ (5:10:4) for 2 min and 50 ml residue washed with $CHCl_3$—MeOH (2:1). Pooled filtrates were dried in a flash evaporator at 30°, dried with toluene–EtOH (4:1) and the crude lipid was partitioned into 240 ml $CHCl_3$—MeOH—$H_2O$ (8:4:3). 150 ml portions of lower phase were then evapd to near dryness and resuspended in $CHCl_3$ and kept in the freezer at −10° under a $N_2$ atmosphere [20].

Total lipids were fractionated into neutral plus glycolipid and PL by chromatography on a silicic acid column [21]. Individual PL from these fractions were separated by 2-D TLC on Si gel with $CHCl_3$—MeOH—7N $NH_4OH$ (115:45:7.5) and $CHCl_3$—MeOH—HOAc—$H_2O$ (170:30:25:6) as 1st and 2nd solvents. Individual spots from TLC plates were scraped into test-tubes and quantitatively analysed for Pi using the method of ref. [22].

All observations are the mean of 4 replications.

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