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MALIC ACID EXUDATION AND PHOTOSYNTHETIC CHARACTERISTICS IN *CICER ARIETINUM*

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Key Word Index—*Cicer arietinum*; Leguminosae; chickpea; malic acid; exudation; photosynthesis.

Abstract—Malic acid exudation and photosynthesis rate were determined in chickpea. Malic acid synthesis and exudation are temperature- and growth stage-dependent. Most of the malic acid is synthesized in glands which also have most of the PEP carboxylase activity. The rate of photosynthesis is comparable to other C_3-plants and the dark CO_2 fixation rate is very low. *Cicer arietinum* cannot be considered a CAM or CAM-like plant.

INTRODUCTION

Sahasrabuddhe [1] reported the occurrence of malic acid in the leaves of *Cicer arietinum* and the influence of water availability on this acid [2]. *Cicer arietinum* is usually grown as an unirrigated crop during winter but it experiences dry weather and relatively higher temperatures (above 30° maximum and above 15° minimum) at the time of fruit development. Recently Santakumari *et al.* [3] described this plant as CAM-like.* However, the presence of PEP carboxylase was reported earlier [4]. Initially we observed droplets containing malic acid on the surface of leaves and fruits. Therefore, we decided to assess the adaptive value of this acid.

RESULTS AND DISCUSSION

Exudation of acid

The amount of titratable acidity increased from the vegetative to the reproductive stage (Table 1); the maximum amount was present in the fruit wall of young fruits. At all stages of growth, except the seedling stage, 78–95 % of the total acid was present on leaf surface and could be washed off.

Table 1. Malic acid content on the surface of leaves and fruit wall of chickpea at different stages of growth

Growth stage	neq/g dry wt
Vegetative	5 (±0.15)
Preflowering	9 (±0.20)
Flowering	12 (±0.32)
Pod setting	19 (±0.14)
Seed development	15 (±0.25)
Young pods (fruit wall)	48 (±0.35)
Mature pod (fruit wall)	22 (±0.22)

The effect of temperature on exudation was studied by placing shoots at different temperatures in the dark with and without brushing leaves. The amount of malic acid increased 2.5-fold with increasing temperature from 5° to 30°. The latter temperature was optimal for malic acid exudation. There was almost no effect of light intensity. In addition, a diurnal analysis of malic acid at 3 hr intervals showed that the maximum amount was reached by 18.00 hr and it stayed as such throughout the night and next morning until it was washed off by water. The maximum exudation from leaves and fruits was 9 neq/g dry wt and 18 neq/g dry wt, respectively. Thus accumulation on the surface is far below the amount of acid accumulation in CAM plants. In nature dew falls on leaves and the wind disperses the acid. In the absence of dew, the acid partially crystallizes on the leaf or fruit wall surface. The malic acid content was studied in eleven mutants obtained from the cultivar JG 62. All mutants and the parental type had the same behaviour and one mutant M 119 maintained a lower acid content than the parent.

Photosynthesis rate and the site of synthesis

The rate of photosynthesis was typical of C_3-plants. The rate of dark CO_2 fixation was 0.5–1 % of the photosynthesis rate. Furthermore, the activities of RuBP carboxylase and PEP carboxylase were determined to evaluate the role of CAM-like or C_4-type behaviour. As most of the acid from leaves or fruit wall could be washed off, the surface morphology of leaves and fruit wall was examined. Both had glands which could be removed by a brush. The activity of RuBP carboxylase, determined in leaves and fruit wall with and without glands, ranged from 110 to 272 μmol CO_2/g fr. wt/hr. There was only a slight loss in activity on brushing away the surface glands (Table 2). The activity of PEP carboxylase was very low compared to RuBP carboxylase and 80 % or more was removed by brushing off the glands (Table 2). Therefore, most of the PEP carboxylase activity is present in the glands. Furthermore, there is no evidence that the malic acid is reutilized by decarboxylation. We can therefore conclude that it would be inappropriate to call this plant CAM or CAM-like.

*Abbreviations: neq, nanoequivalent; CAM, crassulacean acid metabolism; PEP, phosphoenolpyruvate; RuBP, ribulose bisphosphate.

Table 2. The RuBP carboxylase and PEP carboxylase activities of leaves and fruit wall with and without glands of chickpea

Growth stage	Normal (with glands) (μmol CO_2/g fr. wt./hr) RuBP carboxylase	PEP carboxylase	Brushed (without glands) (μmol CO_2/g fr. wt/hr) RuBP carboxylase	PEP carboxylase
Vegetative	260	2.55	172	0.43
Preflowering	206	1.40	203	0.35
Flowering	220	1.50	217	0.35
Pod setting	272	0.71	203	0.15
Grain development	216	0.74	180	0.05
Young pods (fruit wall)	132	1.25	98	0.05
Mature pods (fruit wall)	110	1.70	68	0.05

EXPERIMENTAL

Plants of chickpea (*Cicer arietinum* cv JG 62) were sown on 28 October and 25 November 1979 in the field following recommended agronomic practice. At sampling, 4–5 fully expanded top-most leaves of a branch were taken. Twenty leaflets from these leaves were randomly selected and thoroughly washed in 25 ml distilled water. A 10 ml aliquot of this washing was immediately titrated against 0.01 N NaOH using phenolphthalein as indicator. There were three replicates for each estimation. When the exudation during the day or night was estimated, a group of plants were washed by sprinkling water at the start of the experiment. The effect of temperature was studied by placing shoots of the plant in a BOD incubator at specified temperatures. The importance of glands was examined by brushing the leaf or fruit wall surface using a fine brush. The frequency of gland per unit area was monitored under a microscope to ensure removal of glands. The rate of photosynthesis and dark CO_2 fixation rates were determined following the method of ref. [5]. The activities of RuBP carboxylase and PEP carboxylase were determined according to ref. [6].

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