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# ALKALOID CONTENT IN VARIOUS CHEMOECOTYPES OF *GLAUCIUM FLAVUM* FROM ISRAEL

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**Key Word Index**—*Glaucium flavum*, Papaveraceae; sea poppy, alkaloids, glaucine, isocorydine, halophytes, chemoecotypes

**Abstract**—The inter- and intravariability of various populations of *Glaucium flavum* in Israel were investigated The populations examined exhibit large variations in salt resistance as well as in alkaloid composition and content, and thus seem to constitute separate chemoecotypes Effects of salinity on alkaloid content were equivocal. in some populations salinity reduced the alkaloid content, whereas in others it had no effect. One population of *G flavum* contained no detectable amounts of glaucine, another had an average glaucine content of over 3 6%. The high alkaloid content makes such plants economically attractive for selection of high-yielding genotypes for glaucine and/or for isocorydine production

## INTRODUCTION

*Glaucium flavum* is one of the common plants growing along the coasts of the Mediterranean basin and Western Europe Plants of this species grow abundantly also in the inland continental habitats of Central and Western Asia. In Israel, *Glaucium* appears in scattered stands along the Mediterranean coast from the high coast of Herzylia, in the Tel Aviv region, up to Achziv in Western Galilee. In the past *Glaucium* was much more common along the coastal plain of Israel and its distribution reached the Gaza area However, in recent years its distribution has declined, mostly because of human activity

The scattered distribution pattern of *G flavum* along the coast of Israel raised several questions. Some of them are of a phytogeographical and of a genecological nature and include the question of the species origin, the time of its appearance in Israel, the efficiency of the geographic barriers which exist between the various populations and the state of its taxonomic and ecophysiological evolution The other problems which arise have practical implications For example, do the various populations of *Glaucium* differ in composition and what are the alkaloids which accumulate in the various local populations of *Glaucium* in Israel?

At least 18 different alkaloids, with a very similar structure, are reported in *G flavum* [1, 2] Moreover, different chemoecotypes were distinguished previously in plants from Iran [3, 4]. These chemoecotypes vary in alkaloid content and composition, with some of the populations containing specific alkaloids which are missing from the others.

From previous experience we know that exposure of various species of plants to salt-stresses has a stimulative effect on the production of their secondary metabolites [5, 6]. Thus, as salinity is one of the major selective factors in coastal habitats, the content of glaucine [7, 8] in *G flavum* plants which we have collected at different sites in Israel and later grown in the laboratory under different salt stresses was investigated

## RESULTS AND DISCUSSION

Irrigation of *G. flavum* plants with salt solutions affected their growth and constitution. Considerable differences were observed between the various populations. The C population showed the highest survival in the high salt treatments, whereas the Sh population was the most sensitive to salinity

Analyses of the alkaloid content of leaf-extracts taken from wild plants showed that glaucine (5,6,6a,7-tetrahydro-1,2,9,10-tetramethoxy-6-methyl-4H-dibenzoquinoline) was present in all populations examined except Sh. The concentrations of glaucine varied greatly among individual plants and among populations (A 3.7% ± 0 7, C 1 8% ± 1.0, S 2 1% ± 0.2 dry wt) Similar variations were also detected in the content of the other alkaloids (Fig. 1)

The content of glaucine in plants of population A was the highest among all populations investigated In plant material which was collected in the field, the average concentration of glaucine was 3 6%. Concentrations of up to 5% (dry wt) were not uncommon among individual plants In the laboratory grown plants, the glaucine content of plants of the same population, was significantly higher with an average value of 5%

The Sh population contained no glaucine, the major alkaloid being isocorydine (1,2,10-trimethoxy-6a-aporphin-11-ol) with a mean content of 3 2% ± 0.75 dry wt.

Two additional alkaloids appeared in Israeli *Glaucium* plants in a scattered pattern and in low concentrations They were identified as isoboldine ($C_{19}H_{21}NO_4$, 5,6,6a,7-tetrahydro-1,10-dimethoxy-6-methyl-4H-diben-

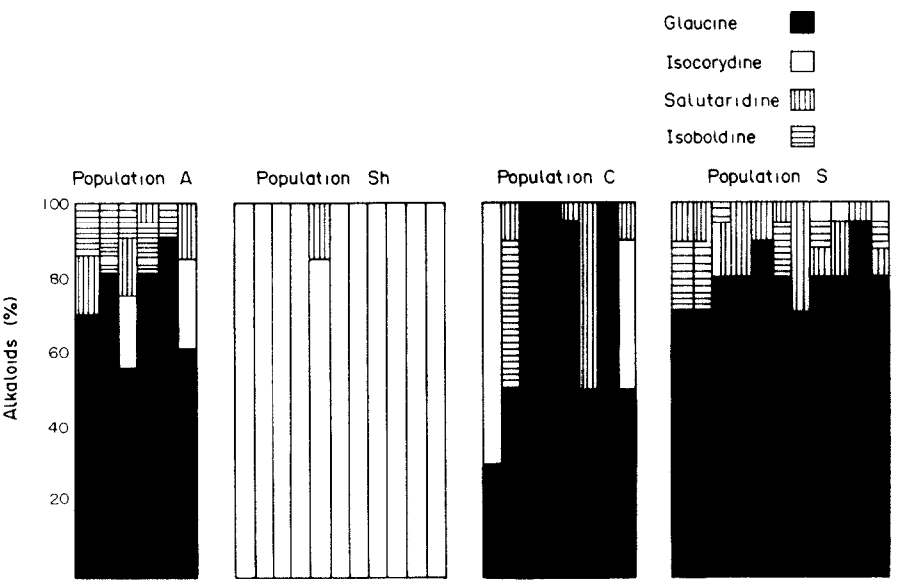


Fig 1 Relative content (% total alkaloid content) of isocorydine, isoboldine, glaucine and salutaridine in different specimens of each of the four examined populations of *Glaucium flavum* (A, Sh, C and S) Plant material was collected in the field Each sub-column represents the results from one plant, whereas all plants of each population are grouped together in order to give the intrapopulation variability

zoquinoline-2,9-diol, $M_r$ 327: mp 178–180°) [9] and salutaridine ($C_{19}H_{21}NO_4$, 5,6,8,14-tetradehydro-4-hydroxy-3,6-dimethoxy-17-methylmorphinan-7-one; $M_r$ 327, mp 197–198°) [10] The former alkaloid has been extracted before only from *Croton* or *Papaver* [11] but not from *Glaucium*

The yield of glaucine in laboratory-grown plants was, in many cases, higher than that from plants collected in the field On the other hand, exposure of the plants to salt stress had an extremely variable effect In most cases it reduced the glaucine content in the plants of the moderate sodium chloride treatments Nevertheless, salinity tended to raise the content of glaucine in the high-salt plants (Fig 2) A similar effect of NaCl on the content of isocorydine and of salutaridine was observed in plants of population C, but not in plants of any of the other populations investigated Only plants from population S were unaffected by the salt treatments

The appearance of apparently new ecotaxa within populations of *G. flavum* in Israel could be the outcome of two factors It might be a consequence of the introduction of these plants from different sources It might also be a result of local selection pressures with the consequent change in genetic constitution and in gene frequency of the various plant populations Some of the changes are apparently adaptive, whereas other characteristics might be by-products of one of the main evolutionary processes

The plants of all four populations of *G flavum* which were investigated look, superficially, very much the same Only a few specimens had some peculiar features which enabled the observer to distinguish them from the others However, careful examinations in the laboratory show clearly that the plants differ in several basic, chemical and physiological characteristics

The appearance of such inherited characteristics, together with some morphological features and the intra- and interpopulation variations in the chemical constitution, indicate that the various populations of *G flavum* in Israel are in an active evolutionary process of ecotypic differentiation and, therefore, justify their classification as distinct chemoecotypes The fact that some of the populations of this plant in Israel have an extremely high content of alkaloids—over five times more than other populations of *Glaucium* in other countries—provides another indication that these plants are at an active state of evolution

It is commonly accepted that environmental stresses tend to raise the content of alkaloids in plants [1, 2] Apparently this is not the case in *G flavum*, where more alkaloids accumulated under the luxurious growth conditions of the laboratory, rather than under the water and salt stresses of the natural habitats Thus, accumulation of such alkaloids in *Glaucium* would appear to be the result of a constructive metabolic pattern rather than that of a catabolic one

The question as to whether the different populations of *G flavum* in Israel are indigenous and have originated from one source or whether they had been introduced several times from several sources remains open However, the striking similarity which was described between one of the populations of Caesarea and some populations near Naples, Italy [12] hints that both possibilities may hold true concomitantly

## EXPERIMENTAL

Plant material for analysis was collected either in the field or in the Tel Aviv University Botanic Gardens where plants from the various populations were cultivated Care was taken to grow only plants from a well defined origin, *viz* Achziv—A, Caesarea—C, Sidni-Ali—S, Shiqmona—Sh The plants were grown either in garden beds, or in sand culture, and irrigated with Hoagland's nutrient sol Plants were irrigated every second

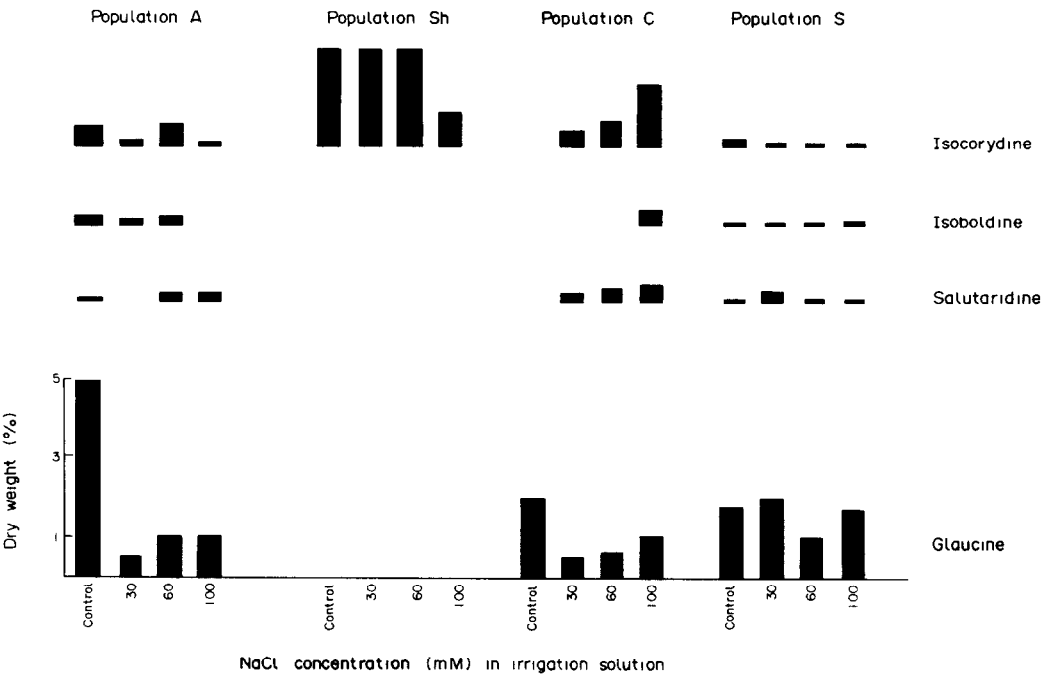


Fig. 2 Effects of irrigation with NaCl on the average alkaloid content (% dry wt) in the four investigated populations of *Glaucium flavum* Plants were grown in the laboratory

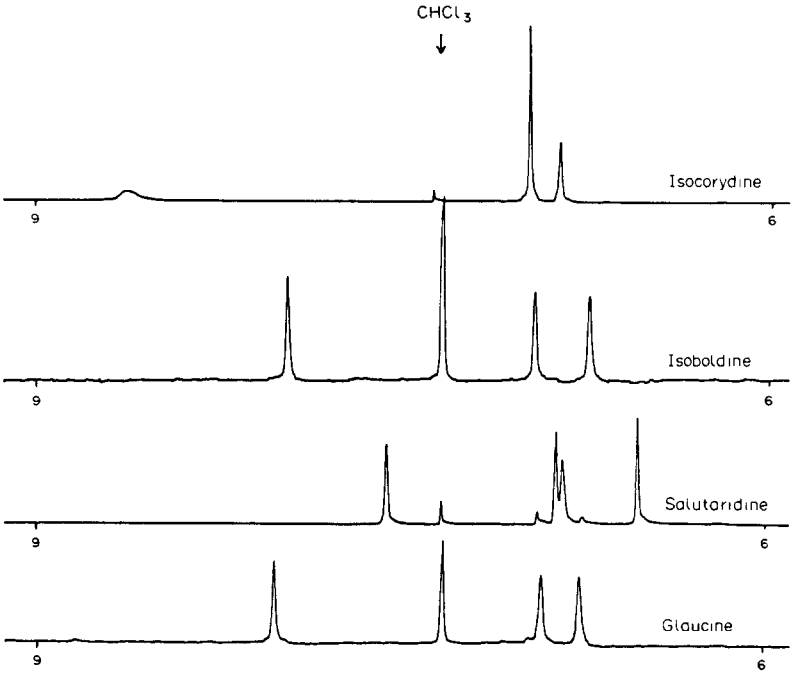


Fig 3 $^{1}$H NMR spectra of the main alkaloids of *Glaucium flavum* (Expansion of the aromatic-H region)

day with the nutrient solution to which various concns of NaCl were added

Alkaloids were extd as follows Leaves were freeze-dried, ground, wetted with $NH_4OH$ (15% v/v), extd $\times 4$ with $CHCl_3$, extd with 5% v/v $H_2SO_4$, extd $\times 3$ with petrol and finally extd again with $CHCl_3$ Identification of alkaloids was achieved by NMR and MS, as well as by mp, the results were compared with the lit NMR spectra were measured at 90 or 270 MHz Mps are uncorr Quantitative determinations were made following extn of leaf samples of 10 g dry wt

For each of alkaloid ext an NMR spectrum was taken Resonances at $\delta$H 6–9 ppm (Fig 3) were compared with those of the pure alkaloids The quantitative composition was calculated from the area ratios of the non-overlapping peaks for the four alkaloids Glaucine was determined from the proton band at $\delta$H 8 09 ppm, isocorydine at $\delta$H 8 83 ppm, isoboldine at $\delta$H 8 01 ppm and salutaridine at $\delta$H 7 65 ppm

## REFERENCES

1 Castedo, L, Suau, R and Mourino, A (1976) *Tetrahedron Letters* **6**, 501
2 Shafiee, A, Ghanbarpour, A, Lalezari, I and Lajevardi, S (1979) *J Nat Prod* **42**, 174
3 Lalezari, I, Shafiee, A and Mahjour, M (1976) *J Pharm Sci* **65**, 923
4 Shafiee, A, Lalezari, I, Lajevardi, S and Khalafi, F (1977) *J Pharm Sci* **66**, 873
5 Waisel, Y (1972) *Biology of Halophytes* 395 pp Academic Press, New York
6 Gershenzon, J (1984) in *Recent Advances in Phytochemistry* Vol 18 (Timmermann, B N, Steelink, C and Loewus, F A, eds), pp 273–320 Plenum Press, New York
7 Jackson, A H and Martin, J A (1966) *J Chem Soc (C)* 2061
8 Novak, V and Slavik, J (1974) *Czech Chem Comm* **39**, 3352
9 Jackson, A H and Martin, J A (1966) *J Chem Soc (C)* 2061
10 Barnes, A R (1964) *Ann Acad Brasil Cienc* **36**, 238
11 Windholz, M (ed) (1983) *The Merck Index* Merck & Co Inc, Rahway, New Jersey
12 Eisikowitch, D (1980) *Israel J Botany* **28**, 87