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Effect of fertilization on growth and solasodine content of four natural *Solanum sodomeum* L. populations

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Abstract

The effect of cow manure or mineral N fertilization was studied on four populations of *Solanum sodomeum L.* grown in Tunisia. The solasodine content of vegetative and reproductive organs was tested to determine the best fertilizing agent to have an optimal yield of solasodine within the limits of a possible industrial use of this species. The experiment was carried out on two sites: S1 fertilized by manure and S2 enriched by mineral N fertilizer. The site effect on average number of flowers per inflorescence was highly significant. The inflorescences were more vigorous in S1. The two sites produced an equivalent variability in average number of fruits per cym. The expression of the genetic potentialities in the elaboration of average number of ripe fruits per plant was not influenced by the cultivation environment. There was a significant variability in the average weight of ripe fruits between the populations and between the sites. The site S2 was more productive. There was not a significant population effect on average diameter of ripe fruits, but mineral N fertilizer was in favour of a better expression of this character. The variation in average solasodine percentage in fresh ripe fruits due to the environment was significant. The aptitudes to accumulate solasodine in yellow fruits were equivalent to the four populations. The fruits were richer in this active agent in S1.

Key words: *Solanum sodoneum L*, natural populations, Solanaceae, cow manure, N fertiliser, solasodine.

Introduction

The natural resources rich in materials having therapeutic application are extremely important. In Tunisia, a plant naturally abundant, *Solanum sodomeum L.,* can constitute an interesting source of solasodine [1]. This plant is a bush which may grow over 1.5 metre high. The stems are green when young and become dark brown and woody with age. It has evergreen leaves with an edge much teethed. The stem and the leaves are very thorny. These biological characters could be means of adaptation to the dryness in order to reduce the processes of evaporation and transpiration. The flowering period may be at any period of the year. The flowers are grouped into cymose inflorescence. They are gamopetalous, tetracyclical and regular. The calyx is short with five green teeth which persist after fertilisation. The corolla has five dark-violet petals. The stamens with anthers facing inwards are joined to the base of the corolla with the base of their filaments. The ovary has one style and two stigmas that protrude from the corolla about the stamens. The fruits are berries, green coloured when immature but become yellow when ripe. They are richer in solasodine than the leaves and stems [1]. This substance is considered as a raw material for steroidic hormones, an active agent having a remarkable anticancer activity [2, 3], an interesting insecticide property [4] and an important anti-accelerater cardiac action [5]. We have carried out in a previous work a comparative study between four natural populations of this species, cultivated on the same soil enriched with cow manure. We have deducted that the yield of solasodine per plant varies according to the degree of maturity and weight of the fruits, the fruit number per plant and the plant genotype [1]. However, we have to take into account that the elaboration of all the morphological characters remains under the simultaneous action of the genotype and the environmental conditions. For this reason, we have judged that it is necessary to carry out a comparative study on two different sites, to determine the fertilization which permits an optimal yield of solasodine.

Materials and Methods

Biological material: The material included four populations of *Solanum sodomeum L.* genetically defined by their biometric characters [6] and called "Djerba"; "Korba"; "Monastir" and "Sfax" by the names of the regions from where they have been taken. This material has many good advantages: abundance in Tunisia, adaptation to dryness, important vegetative development, richness in solasodine and simplicity of the cultivation conditions.

Methods: The germination of seeds was done in Petri dish on a filter paper soaked with distilled water until the persistence of a meniscus of water. At the beginning of germination, the seeds were scarified and put in a total obscurity in the laboratory temperature during three days. The seedlings with a 1.6 cm height were planted out one by one in pots full of fertile soil. Because of the sudden environmental change, some of the seedlings progressively turned yellow and disappeared. After 65 days, the remaining plants were planted out in the field on two sites: S1 fertilized with cow manure at a rate of about 1 kg m^{-2} and S2 enriched with a mineral N fertilizer (N=335 g kg^{-1}) at a rate of 10 g m^{-2}. The plants were disposed in distant rows with an interval of

Table 1. Characters of *Solanum sodomeum* populations fertilized with cow manure or mineral N fertilizer.

Studied variable / Population	Average flower number /inflorescence		Average fruit number/cym		Average ripe fruit number/plant		Average ripe fruit weight (g)		Average ripe fruit diameter (cm)		Average plant height (cm)		Average branch number/ plant		Average solasodine percentage in ripe fresh fruits	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Djerba	5.220	4.865	2.443	2.166	13.186	12.333	9.025	10.341	2.452	2.675	93.969	105.505	11.030	12.409	0.644	0.418
Korba	5.414	5.000	1.844	1.702	5.125	2.375	8.768	11.200	2.389	2.819	78.380	114.327	6.904	9.364	0.703	0.445
Monastir	4.414	4.256	2.285	2.157	12.866	13.437	11.239	12.241	2.557	2.752	91.354	103.232	10.258	0.013	0.595	0.389
Sfax	4.770	4.481	2.645	2.661	11.833	8.513	10.452	12.240	2.509	2.819	81.142	100.072	13.084	18.090	0.407	0.329

S1 Field treated with the cow manure; S2 Field treated with mineral N fertilizer.

Table 2. Effect of environment and population on the considered characters.

Variation source / Studied variable	Environment effect		Population effect	
	F cal	Significance	F cal	Significance
Flower number/inflorescence	30.830	+	52.330	++
Fruit number /cym	5.000	-	37.143	++
Ripe fruit number/plant during the measurement of solasodine percentage	1.136	-	15.900	+
Ripe fruit weight	27.689	+	10.518	+
Ripe fruit diameter	27.833	+	0.722	-
Plant height	8.315	-	0.457	-
Branch number/plant	14.457	+	15.970	+
Solasodine percentage in fresh ripe fruits	55.683	++	4.408	-
F values	F at 5%: 10.13 F at 1%: 34.12		F at 5%: 9.28 F at 1%:29.46	

- Not significant + Significant ++ Highly significant

Table 3. Test "t" values referring the population effects.

Variable / Population		Korba	Monastir	Sfax
Flower number/inflorescence	Djerba*	1.309-	5.230++	2.938++
	Korba*		5.971++	3.743++
	Monastir			1.412-
	Sfax			
Fruit number/cym	Djerba	6.238++	0.121-	4.735++
	Korba		6.623++	9.959++
	Monastir			5.240++
	Sfax*			
Fruit number/plant during the measurement of solasodine content	Djerba*	12.516++	1.527-	3.018++
	Korba		13.389++	5.960++
	Monastir*			3.537++
	Sfax*			
Ripe fruit weight	Djerba	1.348-	2.697++	3.924++
	Korba*		1.032-	1.425-
	Monastir*			1.001-
	Sfax*			
Branch number/plant	Djerba	0.757-	4.356++	6.284++
	Korba		5.577++	5.864++
	Monastir			11.288++
	Sfax*			

Degree of freedom is superior to 30; *Better phenotypic expression;
-Not significant; ++Highly significant

80 cm between each two plants. The plants were irrigated regularly every three days during three months. From the fourth month, the rain quantity became sufficient to their growth. Thirty plants of each population were tested on each site. The studied characters were as follows: flower number per inflorescence, fruit number per cym, fruit number per plant, ripe fruit weight, ripe fruit diameter, plant height, branch number per plant and solasodine percentage in fresh ripe fruits.

We worked in approximately identical meteorological conditions trying to avoid periods of excessive dryness or heavy rain. In fact, some authors have noted that the degree of glucoalkaloids varies according to the meteorological conditions: a prolonged period of dryness produces an increase in the level of glucoalkaloids, while a period of heavy rain decreases their content. During a 24-hour period, the content increases during the diurnal phase and decreases during the night. The essay has been done on ripe fruits carried by secondary axes of the inflorescence at the same age. In fact, we have demonstrated in previous studies, that the berries carried by the principal axes are not as rich in solasodine as the ones carried by the secondary axes [1].

The extraction of glucoalkaloids has been done by a stabilisation with an acetone-water mixture (3:1) during thirty minutes. The acetonic phase was evaporated and the aqueous phase was lyophilised. After acidic hydrolysis with methanol-HCl (88:12), the enzymatic method was applied to the dosage of solasodine [7, 8].

The analysis of the considered characters was conducted according to a factorial design by the software Statistica (TM). An "F" test [9] was calculated to see if the partial variations attributed to the different measured characters on two sites are significant. The comparison of the means [10] as well as their classification was done by the Student "t" test.

Results

The experiment was carried out on two sites: S1 fertilized with manure and S2 enriched by mineral N fertilizer. According to the "F" values (Table 1), the site effect on average number of flowers per inflorescence was highly significant (Table 2). The inflorescences were more vigorous in S1, and the populations "Djerba" and "Korba" gave highest number of flowers per inflorescence on the two sites.

The experimental sites produced an equivalent variability in average number of fruits per cym (Tables 1 and 2). The number of fruits per cym was highest in "Sfax" (Table 3). The expression of the genetic potentialities in the elaboration of average number of ripe fruits per plant was not influenced by the cultivation environment (Table 1 and 2). The "Monastir", "Sfax" and "Djerba" populations gave more ripe fruits per plant in the same period than the "Korba" ecotype.

The "F" test affirmed a significant variability in the average weight of ripe fruits between the populations and between the sites (Table 1 and 2). We supposed that "Sfax", "Monastir" and "Korba" were better to elaborate this character than "Djerba" (Table 3), and the site S2 was more productive (Table 2). There was not a significant population effect on average diameter of ripe fruits (Table 1 and 2), but mineral N fertiliser was in favour of a better expression of this character.

The recorded differences in average plant height were not significant (Table 2) because of the higher interaction between sites and populations. According to the "F" values (Table 2) the "Sfax" population was best in the edification of average branch number per plant on the two sites (Table 3).

The variation in average solasodine percentage in ripe fresh fruits due to the environment was significant (Tables 1 and 2). The aptitudes to accumulate solasodine in yellow fruits were equivalent to the four populations because the "F" values did not demonstrate an evident variation (Table 2). The fruits were richer in this active agent in S1 when the N nutrition was of organic origin.

Discussion and Conclusions

The plants fertilized with cow manure were more vigorous than those treated with mineral N fertilizer. Some authors mentioned that N fertilizer promotes the vegetative development [11]. On the contrary, a carbonic nutrition promotes flowering. The cow manure increases the content of humus and ameliorates the humus-clay complexes. In the humus, the ratio C/N is constant. The mineral nitrogen results from the degradation of the organic material, either in the first phase at the time of humification or later at the time of mineralization by aerobic and anaerobic bacteria and also actinomycetes and fungi. The first step of mineralization is the liberation of NH_4^+ or ammonification. The following step or nitrification conducts to the formation of nitrate NO_3^-. However, at the time of mineralization the carbonic groups degrade more quickly than the nitrogenous groups. This process causes an augmentation of the C/N ratio, which promotes the flowering development. During degradation of organic material nitrogen is removed from the soil which becomes "poor in nitrogen". That's why some basic leaves of the plants on S1 became yellow. The nitric fertilizer rich in NO_3^- decreases the ratio C/N, and facilitates the potassium absorption, which is the principal factor of endo-cellular diffusion. It also promotes the photosynthesis, decreases the transpiration and reduces the risks of withering; so it's a stimulator of vegetative development.

The fruits were more vigorous on S2 than on S1. The obtained results let suppose that the assimilation of the nitric fertilizer nitrogen makes the synthesis of dipolar amino acids easier, particularly that of tryptophane. This substance is an indol-3-acetic acid precursor or auxin. It has the property to increase cell wall elasticity and plasticity and promotes the elongation of aerial axes. Likely this substance promotes, in an average concentration, the bud neo-formation, development and consequently ramification. The auxin which practically has no effect on the flowering, if not in elevated dose, where it is inhibitive, participates to the pericarp development of fleshy fruits. After pollination, the ovary secretes auxin in high quantities which increases the pericarp tissue proliferation. That is why the berries carried by the S2 plants had a superior diameter and weight compared to the ones produced on field treated with cow manure. However, a study concerning tryptophane and auxin dosages is necessary to ameliorate our knowledge about the type of the best fertilizing agent on the vegetative development and reproduction of *Solanum sodomeum.*

Concerning the effect of the fertilizer quality on solasodine content in ripe fruits, we have thought that the nitrogenous assimilation of the nitric fertilizer would speed up the solasodine biosynthesis and thus increase its yield. According to the results,

S1 was better than field treated with mineral N fertilizer. The alkaloid synthesis in plants is for defence against the harmful action of external agents. However, as our plants were well kept, we can admit that the weak stress reduced the synthesis of this active compound. However, an edaphic, climatic and biological stress study is necessary to choose the best environmental conditions to have a good solasodine yield. Likely, calcium absorption is facilitated by NO_3^- ions. Calcium serves as an antidote towards some mineral poisons (heavy metals) and some organic poisons (oxalic acid). So would it be the cause of a moderate alkaloid synthesis on S2 compared to the other site? Concerning the S1 environment, bacteria would form humus, but this restoration would need time. We can admit that the mineral nitrogen, formed later, would contribute to the synthesis of solasodine which would accumulate in high quantities in ripe berries.

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