Response of woad (Isatis tinctoria L.) to different irrigation levels to optimise leaf and indigo production

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Response of woad (*Isatis tinctoria* L.) to different irrigation levels to optimise leaf and indigo production

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Abstract. *Isatis tinctoria* L. (woad) is a potential new crop for southern European countries as source of natural indigo. Water represents an important factor for woad leaf and indigo production, nevertheless few data are available in this respect. With the aim to assess the crop coefficient (Kc), the seasonal crop water requirement (CWR) and the effects of irrigation on vegetative production and indigo yield, six irrigation levels (T100, T80, T60, T40, T20 that received a seasonal water amount equivalent to 100, 80, 60, 40, 20% of ETc and a rainfed control T0) have been compared in a field experiment. The trials have been carried out in Central Italy during two growing seasons characterized by exceptionally rainy (2002) and dry summer conditions (2003) in comparison with the typical ones. Results outlined differences in the daily maximum evatranspiration (ETc) and in the seasonal CWR that differed significantly between the two years being significant higher (+37%) in the dry than in the rainy season. Kc values ranged from 0.30 to 0.47 in relation to plant development. Leaf dry production and indigo yield were unaffected by the level of irrigation both in 2002 and in 2003. Even if *I.tinctoria* appeared to be drought tolerant, going from T0 to T40 a +16% increment in dry leaf and indigo yield has been observed in the driest growing season. In such conditions it is useless to supply a seasonal irrigation volume over 40%ETc i.e. 1330 m^3 per hectare.

Keywords. Isatis tinctoria – Kc – ETc – Irrigation requirement – Leaf – Indigo – Yield.

La réponse du Isatis tinctoria L. (pastel) à l'irrigation différente nivelle pour optimiser la production de feuille et d'indigo

Résumé. *Isatis tinctoria L. (pastel) est une nouvelle culture potentielle pour les pays européens méridionaux comme source d'indigo. L'eau représente un facteur important pour la production de feuille et d'indigo de pastel, néanmoins peu de données sont disponibles à ce regard. Pour le but d'évaluer le coefficient de culture (Kc), le besoin de l'eau saisonnier (CWR) et les effets de l'irrigation sur la production de l'indigo, six niveaux d'irrigation (T100, T80, T60, T40, T20, qu'ont reçu une quantité saisonnière d'eau équivalente a 100, 80, 60, 40, 20% de ETc, et T0 sans irrigation) ont été comparés. Les épreuves ont été effectuées en Italie centrale pendant deux ans caractérisées par les états particulièrement pluvieux (2002) et secs (2003) en comparaison des états typiques. Les résultats ont décrit des différences dans l'evatranspiration maximum quotidien (ETc) et dans le CWR saisonnier qui a différé de manière significative entre les deux années, étant significatives plus haut (+37%) dans la saison sèche que dans la saison pluvieuse. Les valeurs de kc se sont étendues de 0.30 à 0.47 par rapport au développement du plant. La production de feuilles sèches et le rendement d'indigo n'étaient pas changés par le niveau de l'irrigation en 2002 et en 2003. Même si I.tinctoria a semblé être sécheresse tolérant, allant de T0 à T40 on a observé un incrément de +16% dans le rendement sec de feuille et d'indigo en l'année la plus sèche 2003. En telles conditions, il est inutile d'assurer un volume saisonnier d'irrigation au-dessus de 40%ETc, équivalent à 1330 m^3 par hectare.*

Mots-clés. *Isatis tinctoria – Kc – ETc – Condition d'irrigation – Feuille – Indigo – Rendement.*

I – Introduction

Isatis tinctoria L. (woad) is a potential new crop for southern European countries as source of natural indigo (Gilbert and Cooke, 2001; Angelini, 1999). It is a biennial member of the family *Cruciferae*, cultivated in Europe extensively up to XVIII and then abandoned due to the discovery of indigo by synthetic way (Balfour-Paul, 1998). The blue dyestuff was obtained from the leaves,

by water extraction of their indoxyl precursors, isatans and indican, followed by alkali precipitation of the blue powder (Epstein *et al.*, 1967; Gilbert *et al.*, 2004; Angelini *et al.*, 2007). Recently, there is an increasing demand for natural dyes of vegetal origin, included indigo, as renewable materials for industrial textiles dyeing.

To make possible the re-introduction of this crop into the agricultural systems it is necessary to provide new scientific information regarding the agronomic aspects of its cultivation in order to develop efficient and sustainable cultivation methods. Water represents an important factor for leaf production and indigo yield, nevertheless few data are available in this respect (Sales *et al.*, 2006; Campeol *et al.*, 2006). For an efficient use of water resource, the knowledge of crop coefficient (Kc) in the different plant growth stages, is of vital importance in order to estimate the seasonal crop water requirements (CWR). Therefore, the aim of the present work is to assess the Kc values for this specific crop and location, the crop water requirements and the effects of irrigation on both production quantity and quality.

II – Material and methods

1. Field trials

Trials were conducted during the two growing seasons 2002 and 2003 at the Experimental Centre of DAGA-University of Pisa (Pisa countryside, Central Italy 43°41' N; 10°23' E; altitude 5 m a.s.l). *I.tinctoria* seeds were sown in paper pots on March and incubated in germination cabinets under controlled air temperature (20°C) until transplanting in the field in the spring (8th May 2002 and 18th April 2003). The plants were transplanted at 4th-6th true leaf stage with 30 cm inter-row and 10 cm intra-row distances and a crop density of 330,000 plants ha^{-1}. Soil was a typical Xerofluvent of the low Arno river plain, characterized by a superficial water table 120 cm deep in dry conditions. At the beginning of the experimental season, soil was sampled along the profile and physical and chemical characteristics, as well as wilting point and field capacity were measured (Table 1).

Table 1. Chemical and physical characteristics of the soil used for the field trial in 2002 and 2003. Soil was sampled at 20 cm depth in February before planting. Bulk density was averaged on 0-30 cm soil layer.

Parameter	Unit	2002	2003
Sand (2-0.05 mm)	%	36.8	25.8
Silt (0.05-0.002 mm)	%	45.5	46.2
Clay (<0.002 mm)	%	17.7	28.1
pH		8.0	7.9
Organic matter	(%)	1.7	1.3
Total nitrogen	(g kg^{-1})	1.2	1.2
Available phosphorus	(mg kg^{-1})	4.8	15.3
Exchangeable potassium	(mg kg^{-1})	112.2	105.8
Field capacity	% weight	21.0	21.5
Permanent wilting point	% weight	9.6	9.6
Bulk density	g cm^{-3}	1.3	1.3

Fields had been previously cultivated with wheat, and soil was ploughed to a depth of 35 cm in November 2001 and 2002. Ploughing was followed by a superficial disk harrowing in March to a fine tilth to prepare the sowing bed.

Throughout the two experimental periods plants were maintained under identical fertilisation conditions. Mineral fertiliser was applied at pre-planting at rates of 100/100/100 kg ha^{-1} of N/P_2O_5/K_2O. Further 50 kg N ha^{-1} were supplied after the first and the second harvest of the leaves.

Weeds were mechanically controlled by hand weeding. Diseases and insects were kept controlled using commercial pesticides.

2. Irrigation treatments

During the first week of growth in the field, water was supplied in equal amounts to all plots to facilitate post-transplanting recovery. Subsequently, six irrigation levels (T100, T80, T60, T40, T20 that received a seasonal water amount equivalent to 100, 80, 60, 40, 20 % of ETc and a rain-fed control T0) were compared in a randomised block design experiment with four replications. Each plot was 6 m² size with 199 plants per plot. The crop evapotraspiration (ETc) during the growing season was estimated by two microlysimeters while monitoring the climatic parameters and the phenological crop development (Bertolacci and Megale, 1991).The microlysimeters consisted of two prismatic containers (1.20 m x 1.20 m x 0.50 m deep) buried in the soil within the crop layout, leaving two centimeters emerging from the ground. Plants growing in the inside area were therefore perfectly integrated in the crop, thus avoiding advection. The portion of crop confined in the microlysimeters was water fed from a proper artificial water table, placed at the bottom of the containers that were equipped with an automatic device for management and control. The device ensured prompt water replenishment for daily implementation of the automated drip irrigation system, in order to deliver water to the crop at a rate matching water consumption, i.e. water amounts equivalent to 100% ETc rate. Daily meteorological data and daily ETc data were automatically collected and recorded. The water was delivered daily by an automated drip irrigation system equipped with a pressure-compensated and non-leakage dripper line, with emitter flow rate of 2.3 l·hr^{-1} and emitter spacing of 30 cm. To calculate the reference evapotranspiration (ET0), climatic parameters were monitored by a meteorological station and daily measures taken by a Class A pan evaporation placed near the experimental field. ET0 was estimated by the following equation: ET0 = Kp Epan [ET0 = evapotranspiration for grass reference crop, mm day^{-1}; Kp = pan coefficient by Doorenbos and Pruitt, 1977; Epan=pan evaporation, mm day^{-1}].

ETc was calculated by adding any rainfall of significance to the microlysimetric daily water requirement. Therefore, ETc represents the maximum crop evapotraspiration. The ratio between ETc and ET0 within time intervals gives the crop coefficient Kc (Kc = ETc/ET0).

The seasonal crop water requirement (CWR, m³ ha^{-1}), ETc (mm day^{-1}) and Kc were evaluated.

3. Environmental parameters

Changes in air temperature, rainfall, global radiation and Photosynthetically Active Radiation (PAR) were recorded throughout the growing season by using a weather station, properly equipped to the purpose. Cumulative sums of PAR (mE m^{-2}; 1E = 1 moles of photons) and global radiation (KJ m^{-2}) on hourly and daily basis from sowing to harvesting were calculated by a data logger Campbell CR10X. Sensors were mod. Rg19 by Silimet Quantum Sensor system.

4. Plant productive determinations

During the crop cycle the vegetative crop development was followed. Plants were harvested by cutting them at 2 cm above soil level when the diameter of the leaf rosette reached 30 cm and 25 cm plant height. Subsequent harvests were taken when inter-row closure was complete and the same plant height had been regained. Plants grown on the same area were harvested four times in each season: in June (1st harvest), August (2nd harvest), September (3rd harvest) and October (4th harvest). Production measurements (plant fresh and dry yield) were performed on total plot area, excluding the outer rows. Fresh weight was measured, and plants subsequently allowed to dry, first in a greenhouse and later into a ventilated oven at 80°C, for dry weight determination. Measurements made on individual harvest (leaves and total fresh and dry plant yield in t ha^{-1}) were summed to estimate crop seasonal yield.

5. Leaf indigo quantification

To quantify indigo yield from plant samples a modification of Stoker *et al.* (1998) method was adopted for the determination of leaf total indigo. From each plot, 10 medium-sized leaves were randomly chosen. Leaf disks (1 cm) from the middle part of the leaves were taken, weighted and put into a glass tube. 2 ml of deionised water was added and samples were incubated in a boiling water bath for 5 min. Then, the extracts were rapidly cooled to 25°C on ice before removing the leaf discs. 200 µl of a saturated solution of $Ca(OH)_2$ was added and the mixture was aerated for 30 sec at least. An aliquot (500 µl) was transferred into a clear tube and 30 µl 19% HCl were added and the solution gently shaken. Further 2 ml of deionised water and an equal volume of ethyl acetate were added to the solution and shaken to allow a complete partition of the two phases. The blue upper phase of Ethyl Acetate was taken and the indigo concentration of each sample was determined from their absorbance at 600 nm by using an algorithm previously obtained. The measured absorbance was plotted against a calibration curve obtained solving synthetic indigo from Sigma in Ethyl Acetate and measuring the absorbance of a series of diluted solutions.

6. Statistical analysis

All measured and derived data were analysed separately for each year by analysis of variance (ANOVA) using the CoStat software, version 6.201. In all cases, means were separated on the basis of least significant difference (LSD) only when the F-test of the ANOVA treatment was significant at the 0.05 or 0.01 probability level (Gomez and Gomez, 1984).

III – Results and discussion

1. Weather conditions

Total rainfall per month and monthly mean air temperature in 2002 and 2003 are presented in Figure 1. The two growing seasons were characterised by contrasting rainfall distributions during spring and summer in comparison with long-term trend. Considerable variability in rainfall amount and distribution was observed between the two years (468 mm and 59 mm from April to October in 2002 and 2003 respectively) as well as in comparison with the typical long-term trend (426 mm from April to October). In particular, 2003 was characterised by a very dry summer, with rainfall amount significantly lower than the previous year and the typical one. Summer 2002 (June to August) was exceptionally rainy, with a total rainfall of 164.4 mm against 6.3 mm in 2003 and 113.4 mm in the long term.

Mean air temperatures showed the typical long-term trend. Mean monthly temperatures increased from March to the end of July, with a decreasing trend observed thereafter. The 2003 peak value was higher than the 2002 one (33°C vs 29 °C respectively). In particular, higher mean summer temperatures were due to higher maximum temperatures. Cumulative daily PAR (Σ mean values per month in mE m^{-2}) and cumulative Global Radiation (Σ mean values per month in KJ m^{-2}) are reported in Figure 2. PAR showed the typical increasing trend from April to July thereafter it decreased slowly until September. In 2002 weather conditions were unstable throughout summer, with no sustained periods of high irradiance. Global Radiation and PAR summer 2002 values were lower than 2003 ones.

In Figure 3 the trend of ET0 daily values from April/May to October 2002 and 2003 is reported. The values recorded are those typical of the Tyrrhenian coast with peak values at the end of spring and summer when dry windy and sunny conditions occurred. ET0 values in 2003 were always higher than 2002.

2. Seasonal crop water requirement (CWR), crop evapotraspiration (ETc) and crop coefficients (Kc)

The seasonal CWR from April/May to October/November differed significantly between the two years being significantly higher (+37%) in 2003 than in 2002, the wetter season (3327 vs 2080 m^3 ha^{-1}). The seasonal trend of ETc calculated in the two years, is showed in the Figure 4.

To evaluate the ETc and the crop coefficients in the different stages of development, it's useful to keep in mind the phenological development of *Isatis tinctoria*. This species produces an increasing number of leaves organized in shape of a rosette up to when its diameter reaches the size of 20 cm, to pass then to a phase of prevailing growth of the leaf laminas reaching at maturity a plant rosette diameter over 30 cm. Subsequently to the first cutting, the plant develops new leaves reaching again the same diameter. The leaf production after every cutting has the tendency to decrease during the season, mainly as consequence of the diminution of day-length, PAR as well as air temperatures.

The ETc values were different in the two years according with the different trend of the ET0. During the growing season 2002, the medium values of ETc fell in the range 1.3-2.3 mm/day, except in the last month of the season, when they decreased to 0.5 mm/day. This behaviour can be explained by the unusual 2002 cloudy and rainy summer conditions. In particular the abundant rainfalls brought frequently the soil to the field capacity, so plants had much available water and consequently the water supply to the microlysimeters was limited. On the contrary, the 2003 ETc trend was much more irregular than 2002, with the maximum values of 4 mm/day, due to the high sunshine and temperatures during summer with the minimum values immediately after each harvesting.
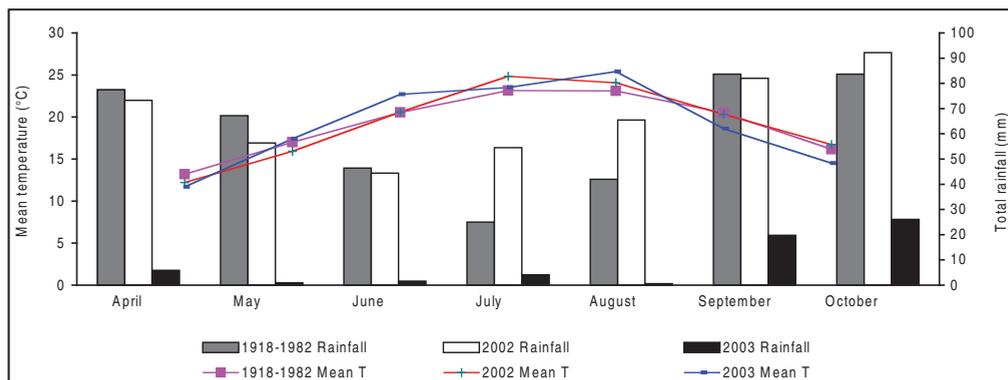

Figure 1. Total rainfall (mm) and mean air temperature (°C) from April to October in 2001 and 2002 growing seasons in comparison with long term 1918-1982 data for the same site.
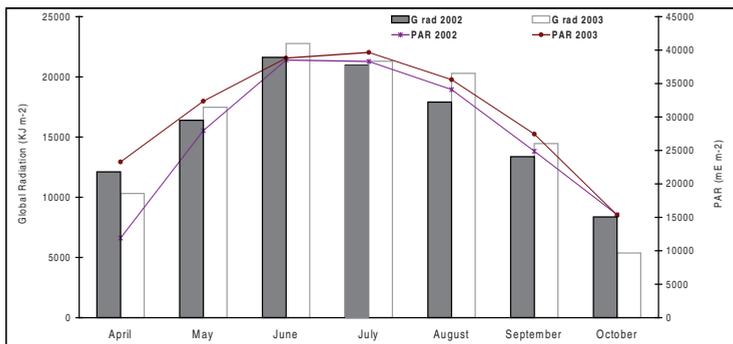
Figure 2. Mean monthly values of Global Radiation (KJ m^{-2}) and Photosynthetically Active Radiation (PAR mE m^{-2}; 1E = 1 moles of photons) measured from April to October in 2002 and 2003 growing seasons.

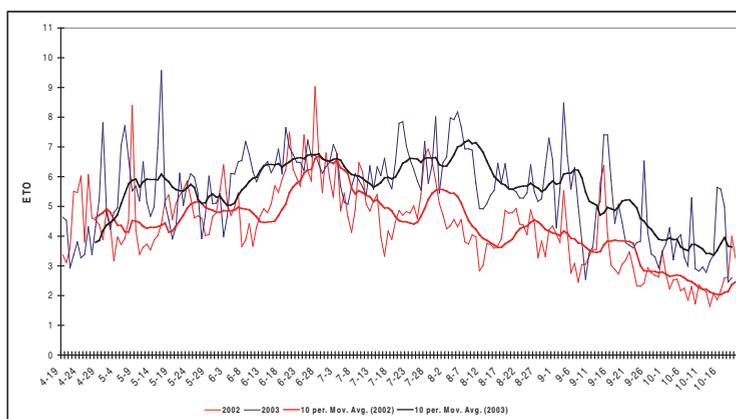**Figure 3. Trend of ET0 (mm/day) daily values from April/May to October 2002 and 2003. Data averaged every 10 days (moving average) represented by the bold lines.**

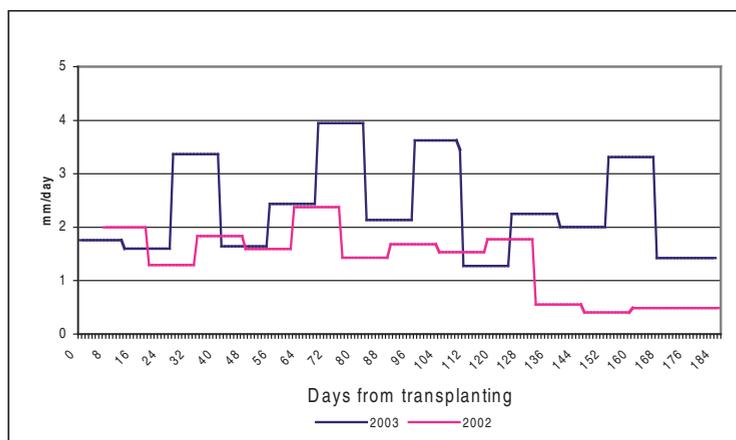**Figure 4. ETc mean values (mm /day) in 2002 and 2003 seasonal course. Values were averaged over 14 days.**

The Kc values measured in the two experimental seasons are illustrated in Figure 5. Kc values were more affected by crop stage of growth than by the contrasting climatic conditions observed between the two years. As expected crop coefficients varied by crop stage of growth. When the crop is fully developed Kc reached the maximum values, whereas in the initial period of vegetative growth and after each harvest, the values were low. The coefficients ranged from about 0.30 at the beginning of plant development (C1= plant with leaf rosette with diameter over 20 cm) to 0.47 at full development (=C2 plant with leaf rosette with diameter over 30 cm). The crop has been harvested four times during the growing season therefore the Kc at full plant development varied among the four cutting-cycles. The plants fully developed and ready for the second cutting in August showed the higher crop coefficients (0.46 and 0.47 in 2002 and 2003). Leaf production after every harvest decreased during the season, mainly as consequence of the diminution of day-length, PAR as well as air temperatures. Consequently before the fourth cutting in Autumn the plants showed the lowest Kc values (0.32 in 2002 and 2003) due to the reduced leaf mass development.
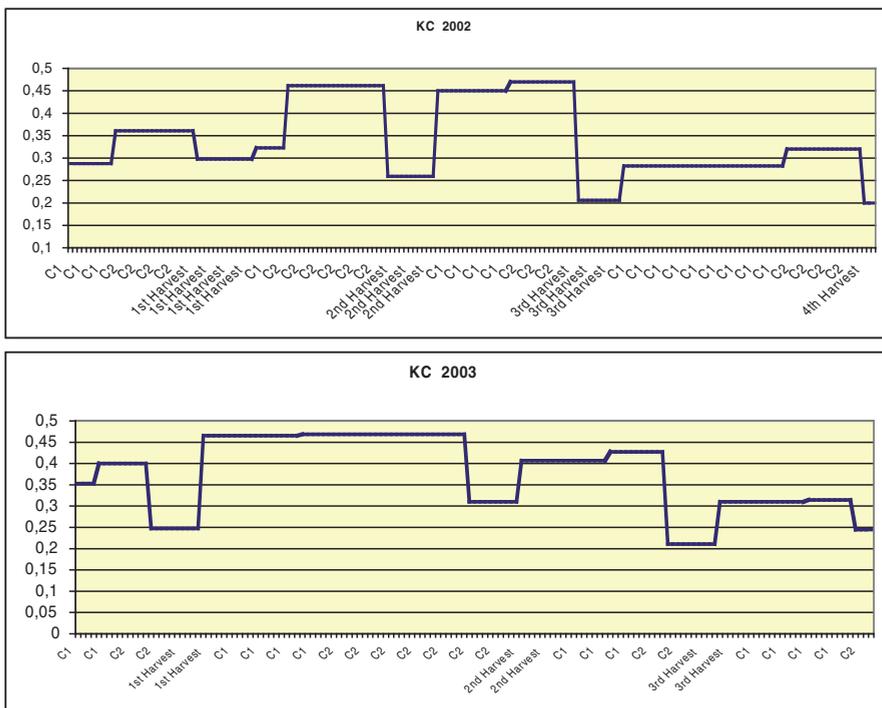
Figure 5. Crop Coefficients (Kc) in the different woad vegetative growth stages in 2002 and 2003 growing seasons. C1= plant with leaf rosette with diameter over 20 cm; C2= plant with leaf rosette with diameter over 30 cm.

Regarding leaf and indigo production, in 2002 the irrigation levels did not affect seasonal fresh and dry leaf production and indigo yield due to the exceptional rainy summer season (Table 3). In the driest 2003 season the irrigation influenced significantly leaf fresh yield and indigo production. The results showed that 40%ETc supply was enough to obtain a fresh leaf yield (t ha^{-1}) higher than T0 and T20. On the other hand, water restitution higher than 40%ETE (T60, T80 and T100) did not give further yield increment (Table 3). Therefore, by a practical point of view, it is useless to supply a seasonal irrigation volume over 40%ETc i.e. 1330 m^3 per hectare.

Table 3. Effect of different irrigation levels on mean fresh (t FW ha⁻¹), dry leaf yield (t DW ha⁻¹) and indigo (kg ha⁻¹) productions on Isatis tinctoria in 2002 and 2003 growing season.

Irrigation Treatments	2002[1]			2003[2]		
	Leaf	Leaf	Indigo	Leaf	Leaf	Indigo
	t FWha⁻¹	t DW ha⁻¹	kg ha⁻¹	t FW ha⁻¹	t DW ha⁻¹	kg ha⁻¹
T_0	94.92	8.90	162.73	52.66 b	8.14	59.62
T_{20}	107.94	10.48	174.12	61.52 ab	8.08	64.90
T_{40}	101.29	8.99	149.76	71.34 a	9.46	69.10
T_{60}	99.70	9.40	139.66	67.04 a	8.64	76.40
T_{80}	101.75	9.77	168.77	65.12 a	8.52	75.70
T_{100}	99.61	9.03	143.24	63.23 a	8.27	82.30
Mean	100.87	9.43	156.38	63.48	8.52	71.34
Significance (LSD)	NS	NS	NS	(10.02)	NS	NS

[1] Harvest dates: 17 June; 29 August; 09 Sept.; 11 Nov. 2002;

[2] Harvest dates: 04 June; 04 August; 11 Sept.; 19 October 2003.

IV – Conclusion

This study provides original information on the water requirements for growing *Isatis tinctoria* under irrigated conditions, particularly with regard to increasing its vegetative growth and the production in its leaves of indigo dyes. Leaf dry production and indigo yield were unaffected by the level of irrigation both under rainy and dry summer conditions. *I.tinctoria* appeared to be drought tolerant in fact it features a deep taproot system and hairy leaves, allowing the plant to withstand water stress. Therefore it is recommended to supply a seasonal irrigation volume not over 40%ETc when severe drought occurred.

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