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Agronomic Potential and Industrial Value of Madder (*Rubia tinctorum* L.) as a Dye Crop*

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Abstract: Madder (*Rubia tinctorum* L.) is a valuable dye crop due to its roots, which are rich in anthraquinone pigments, one of them being alizarin. This study aimed to evaluate the agronomic potential and industrial value of madder plants under rainfed conditions in Southwest Anatolia, Turkey. Three different propagation materials (seeds, seedlings and root cuttings), and 5 different propagation methods (autumn root transplanting, spring root transplanting, autumn seed sowing, spring seed sowing, and spring seedling transplanting) were used in the study. At the end of the 3-year growing period, fresh root yield varied from 1640.1 kg ha^{-1} (in the spring root transplanting) to 4813.2 kg ha^{-1} (in the spring seedling transplanting). As a result, compared to the other methods, spring seedling transplanting had the best performance, producing higher root and dye yields. Of the fresh roots 34.0%-37.5% was dry matter, represented by dry root yields. Although there was no statistical difference between the propagation methods, the roots from the seedlings gave the highest dye content (2.20%). There was a remarkable variability between 1.98% and 3.70% in the dye content of the roots depending on root positions on the main root and growing stages within a year. It was determined that August was the optimum harvest time for obtaining the highest dry matter and dye matter accumulation. Dye matter was accumulated 2.3 times more in the cortex than in the stele, and the highest dye content and cortex ratio were found in the roots in the tertiary position.

Key Words: Madder, *Rubia tinctorum* L., propagation methods, dyeing analysis

Bir Boya Bitkisi Olarak Kökboyanın (*Rubia tinctorum* L.) Tarımsal Potansiyeli ve Endüstriyel Değeri Üzerine Bir Araştırma

Özet: Kökboya (*Rubia tinctorum* L.), alizarin gibi anthraquinone pigmentlerince zengin olan kökleri nedeniyle değerli bir boya bitkisidir. Bu araştırmada, Türkiye'nin Güney Batı Anadolu Bölgesi'nin su ihtiyacı doğal yağışlarla karşılanan tarla koşullarında, kökboyasının tarımsal potansiyelini ve endüstriyel değerini belirlemek amaçlanmıştır. 'Tohum', 'fide' ve 'kök çeliği' olarak üç farklı üretim materyali kullanılmış, 'güz dönemi kök çeliği dikimi', 'ilkbahar dönemi kök çeliği dikimi', 'güz dönemi tohum ekimi', 'ilkbahar dönemi tohum ekimi' ve 'ilkbahar dönemi fide dikimi' olarak beş farklı üretim yöntemi uygulanmıştır. 3 yıllık yetişme sezonu sonunda, taze kök veriminin 1640 kg ha^{-1} (ilkbahar dönemi kök çeliği dikimi) ve 4813.2 kg ha^{-1} (ilkbahar dönemi fide dikimi) arasında değiştiği, en yüksek kök verimi ve boya veriminin bahar dönemi fide dikimi ile yapılan üretimden elde edildiği saptanmıştır. Taze köklerin %34.0-37.5'ini kuru kök verimini temsil eden kuru madde oluşturmuştur. Her ne kadar üretim yöntemleri arasında boya içeriği bakımından istatistiksel bir fark bulunmamış ise de, en yüksek boya içeriği %2.20 ile fidelerden üretilen köklerden elde edilmiştir. Köklerde boya içeriği; ana kök üzerinde kök pozisyonlarına ve yıl içinde büyüme dönemlerine bağlı olarak %1.98 ile %3.70 arasında geniş bir değişim göstermiştir. Kökboyada en yüksek kuru madde ve boyar madde birikiminin olduğu Ağustos ayı, en uygun hasat zamanı olarak saptanmıştır. Kökün korteks (kabuk) kısmında odun (öz) kısmına göre 2.3 kat daha fazla boya birikmiş, tersiyer posizyondaki köklerin daha yüksek boya içeriğine ve korteks oranına sahip olduğu bulunmuştur.

Anahtar Sözcükler: Kökboya, *Rubia tinctorum* L., üretim yöntemleri, boya analizi

Introduction

Synthetic dyes used in textile dyeing generally cause environmental pollution and health problems in humans. Furthermore, many synthetic colorants are classified as toxic when in contact with the skin, and consumers in the EU demand safer textile products, especially for babies and children (Ankliker et al., 1988; Angelini et al., 2003). Therefore, the usage and production of natural dyes produced from plants has become more popular due to the growing awareness about the environment, health

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and the need for alternative crops. The fastness degree of natural dyes obtained from dye plants is also higher than that of synthetic dyes.

Interest in natural dyes, especially from the common madder (*Rubia tinctorum* L., family Rubiaceae), which is the source of 'Turkey-red' dye, has increased considerably in recent years (Derksen, 2001). With different mordants, madder gives quality colors ranging from salmon pink (with copper) to bright red (with alum). When it used without a mordant, it gives coral to rust shades (Dean, 2003).

Madder is a perennial herbaceous plant and produces roots (or rhizomes) used for dyeing. The madder roots continue to grow and spread underground, producing fresh shoots above ground each year. Madder roots are very rich in anthraquinone pigments, one of them being alizarin (1,2 dihydroxy anthraquinone), which has been used for dyeing textiles since 2000 B.C. (Burnett and Thomson, 1968; Angelini et al., 1997).

Madder was one of the most commonly cultivated plants in Turkey during the Ottoman period for the production of hand-woven carpets. It was reported in the Ottoman archives that the primary madder demand of the world was met by Turkey (Sanayi ve Ticaret Bakanlığı, 1991). After the epoch of synthetic dyes in the 19th century, madder cultivation slowed down at first and was later abandoned altogether. Nowadays, madder, like other dye plants, is intensively collected from wild populations, and is considered under threat of genetic erosion. In particular, excessive exploitation of *R. tinctorum* L. has resulted in a dramatic reduction and has even caused complete extinction of natural populations. Recently, there has been rising interest in the cultivation of *R. tinctorum* L. for its protection and increased demand as a source of red dye in textile production. The aim of present research was to evaluate its agronomic potential and industrial value as a dye crop.

Materials and Methods

Field trials

Field trials between 1999 and 2002 under rainfed condition were set up at the Kuleönü Experimental Station (37° 45' N; 30° 33' E; 997 m above sea level) at Süleyman Demirel University in Isparta, located in western part of the Mediterranean region in Turkey. The soil of the experimental field was clay-loam (sand 17%; silt 25%; clay 58; organic matter 1.3%; pH 7.8; total nitrogen 2.1%, P_2O_5 0.2 kg ha^{-1}; K_2O 10.4 kg ha^{-1}). The trends of temperature and rainfall during the 2000-2002 trials are presented in Table 1.

Table 1. Climatic conditions during the trials in 2000, 2001 and 2002.

Months	Rainfall (mm)			Temperature (°C)		
	2000	2001	2002	2000	2001	2002
January	32.8	62.4	22.3	-1.6	4.0	0.4
February	42.0	30.6	10.3	1.9	4.1	6.1
March	43.8	21.0	50.9	4.5	11.0	8.4
April	76.6	57.8	134.6	11.6	11.3	10.2
May	63.3	68.3	45.7	15.4	15.6	15.9
June	16.7	3.3	9.0	22.1	22.0	21.1
July	0.0	5.5	10.6	26.1	25.9	23.7
August	4.7	2.8	9.0	23.6	24.9	22.5
September	9.7	10.4	73.7	18.9	19.8	16.6
October	32.9	0.0	5.2	12.2	13.6	13.1
November	66.4	157.1	38.0	8.8	7.3	8.2
December	39.3	217.8	99.2	3.4	3.7	0.9
Total	428.2	637.0	508.5	-	-	-

Seeds (1), seedlings (2) and root (rhizome) cuttings (3) were used as propagation materials: 1) Madder seeds were collected from plants growing naturally in the Eğirdir and Atabey districts of Isparta in October 1999. Seeds were kept at 4 °C in paper bags for a month, and then directly sown in the experimental plots on November 29, 1999 (autumn sowing), and on March 29, 2000 (spring sowing). 2) Madder roots were freshly collected from plants growing wild in the Eğirdir and Atabey districts of Isparta, and then cut into pieces 15 cm in length and with 4 or 5 nodes. The root cuttings were stored at 4 °C in plastic bags in a refrigerator until transplanting. They were transplanted into the plots on November 29, 1999 (autumn transplanting and on March 29, 2000 (spring transplanting). 3) The seeds were moistened with distilled water for 1 week prior to sowing into pods filled with compost: garden soil: brook sand (1:1:1) in a greenhouse on March 22, 2000. Afterwards, 4-week-old seedlings were transplanted into the plots on April 21, 2000.

The experimental design was a randomized complete block with 3 replications. Row distances of 75 and 25 cm were used. This provided a population density of about 5.3 plants per square meter. Plot width and length were 3 and 4 m, respectively. All plots received 50 kg ha^{-1} N and 50 kg ha^{-1} P_2O_5 each year. The plots were kept weed free by hoeing, and plants were grown without irrigation. Madder roots were dug up on August 21, 2002, when they were 3 years old. Fresh and dry root weights (kg ha^{-1}) were calculated from the 2 middle-row plants of each plot. The roots were ground in a blender and stored in paper bags in a dry place until the dye analysis.

Dry matter and dye matter accumulation

The root samples were collected from 3-year-old madder plants. The collection was done at the beginning of each month in 2002. The roots were classified into 3 groups according to their size: primary roots (>1.5 cm in diameter), secondary roots (0.5-1.5 cm in diameter) and tertiary roots (<0.5 cm in diameter). After drying at 75 °C for 3 days, they were weighed to determine dry matter accumulation (%, in fresh root) and dye matter accumulation (%, in dry root). Total dye content was also performed on 2 main root parts, the cortex and stele, as described by Angelini et al. (1997), with some modifications.

Total dye analysis

After drying, roots were ground in a blender. Fifty milligram ground root samples were fermented for 12 h with 50 ml of distilled water. Later they were kept in a water bath at 70 °C for 1 h, and 50 ml of ethanol was added to the samples. The samples were cooled on a shaker and filtered through a 0.2 mm filter (Millipore). Each filtered sample was brought up to 100 ml with 50% ethanol. One milligram of purpurin ($C_{14}H_8O_5$, Sigma C.I. 58205) and 1 mg of alizarin ($C_{14}H_8O_4$, Fluka 05560) were dissolved in a beaker with 100 ml pure ethanol using an ultrasonic bath. The standard concentration was equal to 2. The absorbance values of all ethanol extracts and standard solution were measured at 450 nm using a spectrophotometer (Perkin Elmer UV/VIS Lambda 20). Total dye content based on alizarin and purpurin standards was calculated using the formula given by Siebenborn et al. (1997):

Total dye content (%) = (100/50) x [(Standard concentration x Sample absorbance value) / (Standard absorbance value)]

Statistical analysis

Statistical analysis was carried out using the MSTAT-C software package (version 2.1, Michigan State University, 1991). When statistically significant treatment differences were detected, means were separated by the LSD at the 0.01 level.

Results and Discussion

The growth and development stages of the madder plants were observed phenologically during the experimental years. The appearance of shoots from the nodes of the roots extending under soil started in the last days of March or the first days of April. After the beginning of the hot and rainy days, especially in May (Table 1), the shoots grew very fast and reached about 2 m long on the ground in June. The madder plants started flowering in the last days of June, began fruiting in the second part of July and August, and totally matured in September and October. It was interesting that while the madder plants from autumn and spring root transplanting and autumn seed sowing started flowering in the first flowering season in 2000, the madder plants from spring seed sowing on March 10 in 2000 and spring seedling transplanting on April 21 in 2000 started flowering in the second growing season in 2001. The growing season of 2002 was characterized by relatively low temperatures and high rainfall during spring and summer, which caused late flowering and maturing. In the first (2000) and second (2001) growing seasons, madder plants produced very thin and weak roots. Therefore, these roots were harvested in the third (2002) growing season.

In madder, the roots are mostly collected from the 3-year-old plants because it is less time-consuming and laborious. In the following years, the old and deep roots of the madder plants begin to decay, which makes the roots poor in dye yield and quality. It has been previously reported (Derksen, 2001) that if madder root were cultivated for 3 instead of 2 years the amount of alizarin increased from 6.7 to 8.7 mg g^{-1}.

The fresh and dry root yields together with dry matter contents in the 3-year-old plants are given in Table 2. Dry matter contents of the madder roots changed from 34.0% (autumn root transplanting) to 37.5% (autumn seed sowing). However, no significant differences were found between the propagation methods. Significant differences in dry and fresh root yields from 3-year-old plants were observed. Fresh root yield varied from 1640.1 (spring root transplanting) to 4813.2 kg ha^{-1} (spring seedling transplanting). Commercial yield, represented by dry yields, varied from 583.1 (spring root transplanting) to 1716.0 kg ha^{-1} (spring seedling transplanting). These results show that approximately 35% of the fresh roots was only dry matter (Table 2).

Angelini et al. (1997) estimated about 3.5 t ha^{-1} of dry root yield from the 30-month-old madder plants grown in Germany, France and Italy. They found that root yield increased from young (5 months old) to mature plants (30 months old), reaching a mean value of 120 g $plant^{-1}$. The yield estimation as 3.5 t of dry root by Angelini et al. (1997) was done in 30-month-old plants on a per plant basis. Therefore, differences in root yields between 2 field trials would result from plant ages and yield estimation methods together with environmental conditions.

Total dye contents and yields in the third growing season are given in Table 3. Among the propagation methods, no significant differences in dye contents were observed. Dye contents changed between 1.86% (18.6 mg g^{-1}) in the spring root transplanting and 2.20% (22.0 mg g^{-1}) in the spring seedling transplanting. According to Derksen (2001), alizarin changes from 6.1 to 11.8 mg g^{-1} root. In this respect, alizarin is the most important part of dye components in madder root.

There were significant differences in the dye yields based on per hectare and per plant, although there were no significant differences in the dye yields based on milligrams per gram dry weight (Table 3). Dye yield varied from 11.0 kg ha^{-1} (spring root transplanting) to 37.7 kg ha^{-1} (spring seedling transplanting), from 205.8 mg $plant^{-1}$ (spring root transplanting) to 706.5 mg $plant^{-1}$ (spring seedling transplanting) dry weight, and from 18.6 mg g^{-1} (spring root transplanting) to 22.0 mg g^{-1} (spring seedling transplanting) dry weight. As a result, compared to the other methods, spring seedling

Table 2. Fresh and dry root yields of *R. tinctorum* L. from different propagation methods.

Propagation methods	Root yield (kg ha^{-1})		Dry matter (%)
	Fresh yield	Dry yield	
Autumn root transplanting	2669.0 b[1]	905.6 c	34.0
Spring root transplanting	1640.1 c	583.1 d	35.6
Autumn seed sowing	3096.3 b	1162.0 b	37.5
Spring seed sowing	1854.4 c	659.2 d	35.0
Spring seedling transplanting	4813.2 a	1716.0 a	35.6
Overall mean	2814.6	1005.2	35.5
F value	187.3**	142.9**	1.32

[1]Values within a column followed by the same letter are not significantly different at the 1% level (LSD test). ** $P < 0.01$

Table 3. Dye content and dye yields of *R. tinctorum* L. from different propagation methods.

Propagation methods	Dye content (%) (Mean ± SE)	Dye yield		
		kg ha^{-1}	mg $plant^{-1}$ dry weight	mg g^{-1} dry weight
Autumn root transplanting	2.12 ± 0.10	19.4 b[1]	363.5 b	21.3
Spring root transplanting	1.86 ± 0.22	11.0 c	205.8 c	18.6
Autumn seed sowing	1.96 ± 0.16	22.5 b	422.0 b	19.6
Spring seed sowing	2.10 ± 0.13	13.8 c	258.6 c	20.9
Spring seedling transplanting	2.20 ± 0.12	37.7 a	706.5 a	22.0
Overall mean	0.93	20.9	391.3	20.5
F value	12.0	99.4**	99.1**	0.93

[1]Values within a column followed by the same letter are not significantly different at the 1% level (LSD test). ** P < 0.01

transplanting gave the best performance, producing higher root and dye yields (Table 3).

The madder roots are made of 2 main parts, the cortex (outer part) and stele (central part). The percentages of cortex and stele in madder roots and their dye contents are presented in Table 4. Madder root was composed on average of 57.7% cortex and 42.3% stele. Dye contents of these main root parts were 3.72% and 1.61%, respectively. Dye analysis, which was performed on different root parts, revealed that dye matter was accumulated 2.3 times more in the cortex than in the stele. These findings were also in accordance with Angelini et al. (1997), who reported that alizarin was accumulated in a larger amount in the cortex (1.9 mg g^{-1} dry root) than in the stele (0.7 mg g^{-1} dry root). The highest dye content (3.82%) and cortex ratio (61.8%) were found in the roots whose thickness was lower than 1.5 cm. In general, tertiary roots were not thicker lower than 1.5 cm in diameter. Therefore, selection should be based on a higher cortex ratio, especially from tertiary positions, which have more dye contents in madder plants (Table 4).

Changes in the dry matter and dye content in the primary, secondary and tertiary roots from 3-year-old plants are given in Table 5. Primary roots were the main roots of the plants, which are generally larger than 1.5 cm in diameter. Secondary roots were side branches of the primary roots, which were generally ranged from 1.0 to 1.5 cm in diameter. Tertiary roots were the youngest ones on the secondary roots, and they were generally smaller than 1 cm in diameter. In the present research, the highest dry matter was accumulated in the primary roots, but the highest dye matter was accumulated in the tertiary roots. The highest dry matter and dye content of these root positions were found in August at the end of the third growing season (Table 5).

Because of the higher dye contents of the tertiary roots, it seems that the younger roots were more dye

Table 4. The percentages of cortex and stele in *R. tinctorum* L. roots and their dye contents.

Root diameter	Ratio in root (%) (Mean ± SE)		Dye content (%) (Mean ± SE)	
	Cortex	Stele	Cortex	Stele
> 1.5 cm	53.7 ± 3.7	46.3 ± 1.9	3.62 ± 0.26	1.69 ± 0.11
< 1.5 cm	61.8 ± 2.9	38.2 ± 2.1	3.82 ± 0.18	1.54 ± 0.14
Overall mean	57.7	42.3	3.72	1.61

Table 5. Changes in dry matter and dye content of *R. tinctrom* L. in the different root positions during the growing season.

Months	Dry matter (% in fresh root)				Dye content (% in dry root)			
	PR	SR	TR	Mean	PR	SR	TR	Mean
January	28.0	26.7	18.3	24.3	2.37	2.72	2.80	2.63
February	27.6	22.2	18.0	22.6	2.14	2.42	2.61	2.39
March	26.1	21.8	16.4	21.4	2.08	2.19	2.21	2.16
April	24.9	20.6	16.2	20.5	2.15	2.42	2.52	2.36
May	25.8	21.9	17.6	21.7	2.43	2.97	3.31	2.90
June	29.4	25.9	23.1	26.1	2.60	3.12	3.36	3.02
July	37.2	35.3	34.0	35.5	2.58	3.30	3.52	3.13
August	39.6	37.5	34.3	37.1	2.68	3.39	3.70	3.25
September	36.7	33.0	30.6	33.4	2.36	3.00	3.43	2.93
October	34.1	30.8	29.5	31.5	1.98	2.54	2.72	2.41
November	33.5	29.3	28.5	30.4	2.21	2.24	3.03	2.49
December	29.6	27.7	22.5	26.6	2.57	2.81	3.07	2.81
Overall	31.0	27.7	24.1	27.6	2.34	2.76	3.02	2.70

PR: Primary root, SR: Secondary root, TR: Tertiary root

rich than the older ones. As a result, the tertiary roots extending in soil should be collected primarily to get more roots rich in dyestuff during the harvest. Significant increases in dry matter from June (26.1%) to August (37.1%) were detected, after which it decreased gradually from August to April (20.5%). Dry matter accumulation in madder plants is generally influenced by temperature during the growth periods. The temperature increases during the summer months increased photosynthetic dry matter production in the madder roots (Table 5).

Changes in the dye contents are of special importance to the quality of the madder roots. In the present study, a near parallel pattern was observed between dry matter accumulation and dye content in roots. Dye accumulation in the roots changed significantly during the growing periods as well as dry matter. A rapid increase in dye synthesis started in April (2.36%) and it reached its maximum level in August (3.25%) without abrupt fluctuation. After August, the dye matter accumulation declined gradually and reached its minimum level in March (2.16%). Dye accumulation patterns in the different root parts were also quite similar to each other, with slight fluctuations as plants develop (Table 5). As well as dry matter, dye matter synthesis was also encouraged by temperature increases after April (Table 1).

Conclusions

Many important factors affect the yield and quality of madder roots. It was determined in this study that seedling transplanting is the most suitable method in madder propagation. The 3-year-old madder plants produced from spring seedlings gave the best root yield (about 4.8 t ha^{-1}) and dye yield (37.7 kg ha^{-1}). This method could be used to extend the growing season by producing seedlings for field transplanting as soon as the danger of spring frosts is over. This procedure also avoids some of the environmental hazards of germination and allows plants to be placed directly into a final spacing. Autumn root transplanting could also be used for practical and easy production in madder. August was determined as the optimum harvest time for obtaining the highest dry matter and dye content.

The tertiary roots containing more cortex and dye content should be collected primarily to get roots, which would give the highest quality.

Our results could be improved by experiments including more genotypes, locations and agronomic applications. However, the information obtained from the present study could be useful in research determining the ideal production methods, the ideal harvest time and plant parts to improve the dye content and dye quality in madder.

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