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Castor oil as an innovative raw material for an energy supply chain in semi-arid environments

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Research highlights

- Renewable energy sources provide an exceptional opportunity for mitigation of greenhouse gas emission and reducing global warming
- Castor plant is an important renewable resource that has a high potential for use as a biorefining feedstock
- I assessed the adaptability of Castor in semi-arid Mediterranean environment and the bioenergetic potential of this crop
- The field trials showed that Castor is a promising crop well adapted to grow in the southern Mediterranean basin
- In the laboratory, the analysis of composition allowed to consider castor capsule residue as a potential substrate useful for anaerobic digestion to produce biomethane.
- These results suggest that further studies are necessary to identify the optimal crop management and, in a biorefinery perspective, other pretreatment methods to enhance the energy conversion.

Abstract

Increasing energy demand and fossil fuel prices together with environmental concerns have motivated scientists to find alternative energy sources. Renewable energy sources hold the key potential to displace greenhouse gas emissions from fossil fuel-based power generating and thereby mitigating climate change. Biofuels are renewable sources of energy with acceptable environmental impacts. Biodiesel is the most common types of biofuels. Bioethanol and biogas are other biofuels that are industrially produced from easily degradable biomass resources.

Castor bean (*Ricinus communis* L.), a non-edible multipurpose oilseed species, is an important renewable resource that has a high potential for use as a biorefining feedstock. Castor is considered to be one of the most promising oil crop, due to its high seed annual yield and its adaptability to semiarid climate and adverse growing conditions. Moreover, it is considered as a second-generation raw material for the production of bioenergy or industrial purposes. Indeed, castor oil can be used for biodiesel production while the main by-products generated in the castor oil production (capsule husks and meal) and the residual biomass, are potentially applicable as feedstocks for advanced ethanol and biogas.

The present research project investigated the adaptability of Castor in semi-arid Mediterranean environment and the optimization of the cultivation techniques. Concerning the bioenergetic potential of this crop, lignocellulosic capsule residue from seed processing was used to produce biomethane via anaerobic digestion process.

Keywords: *Ricinus communis* L., Mediterranean environment, seed yield, oil yield, energy crops, biofuel, biomass, biodiesel, biomethane, anaerobic digestion.

Riassunto

Le energie da fonti rinnovabili offrono un'opportunità eccezionale per limitare le emissioni di gas serra e ridurre il riscaldamento globale sostituendo le fonti energetiche convenzionali (basate su combustibili fossili) e quindi mitigare i cambiamenti climatici. Le bioenergie derivate biomassa organica, si sono affermate sempre più come un'alternativa valida alla produzione di calore, elettricità ed energia per autotrazione.

Le bioenergie contribuiscono a gestire in maniera più efficiente alcuni residui agricoli e allo stesso tempo consentono di valorizzare i sottoprodotti da un punto di vista energetico. Il ricino (*Ricinus communis* L.) è una coltura no food, che ha grandi potenzialità come coltura energetica in quanto fornisce diversi prodotti utili alla filiera bioenergetica: l'olio di ricino può essere utilizzato per la produzione di biodiesel mentre i principali sottoprodotti (capsule e farina di estrazione) e la biomassa residua sono potenzialmente utilizzabili come materie prime per la produzione di biogas.

Lo scopo del presente lavoro è valutare l'adattabilità di questa specie all' ambiente semi-arido Mediterraneo e ottimizzare le tecniche di coltivazione al fine di massimizzare la resa in seme e in olio. Per quanto riguarda il potenziale bioenergetico di questa coltura, il residuo della capsula lignocellulosica proveniente dalla lavorazione dei semi è stato utilizzato per produrre biometano tramite processi di digestione anaerobica.

Parole chiave: *Ricinus communis* L., ambiente Mediterraneo, resa in seme, resa in olio, colture energetiche, biocarburanti, biomassa, biodiesel, biometano, digestione anaerobica.

1 General introduction

1.1 State of the art: Castor oil as an innovative raw material for an energy supply chain in semi-arid environments

Topics discussed in this review include: botanical descriptions, ecology, biotic and abiotic stresses, development of commercial cultivars, agricultural practices, irrigation and fertilization and plant nutrition.

1.1.1 Introduction

Castor (*Ricinus communis* L.) is a non - food, drought resistant, energy crop well known since antiquities, for the peculiar characteristics of its oil, being evidences of castor oil uses since Egyptian times (Scarpa and Guerci, 1982; Franz, 1988; Weiss, 2000). *Ricinus communis* L. is known by several names including: ricino, castor, tártago, higuerilla, mamoneira, mamona, palma christi, higuereta, castor bean, castor-oil plant.

Castor is a member of the Euphorbiaceae family that is found across all the tropical and semi-tropical regions of the world (Weiss, 2000). It is cultivated in 1.5 million ha worldwide, mainly in India, China, Brazil, and Mozambique (Fernández-Martínez and Velasco, 2012). Castor seed contains between 40% and 60% oil, which is rich in triglycerides, mainly ricinolein. This oil is highly viscous, its coloration ranges from a pale yellow to colorless; it has a soft and faint odor and a highly unpleasant taste. Castor oil dissolves easily in alcohol, ether, glacial acetic acid, chloroform, carbon sulfide, and benzene. It is made up of the following fatty acids 91–95% ricinoleic acid, 4–5% linoleic acid, and 1–2% palmitic and stearic acids (Beltrão, 2005). The castor bean oil contains 1–5% of a cytotoxic protein, named ricin, one of the most potent and deadly plant toxins known (Aslani et al., 2007). Castor oil is non edible and has been used almost entirely for pharmaceutical and industrial applications.

The oil extracted from the castor bean already has a growing international market, assured by more than 700 uses, ranging from medicines and cosmetics to substituting petroleum in the manufacturing of plastics, lubricants and biodiesel, (Azevedo et al., 1997; Freire, 2001). Biodiesel are becoming big policy and big business as countries around the world looking to decrease petroleum dependence, reduce greenhouse gas (GHG) emissions in the transportation sector, and support agricultural interests (Berman et al., 2011). Biodiesel is a mixture of monoalkyl esters of vegetable oils or animal fats, which may be produced via a transesterification reaction (Demirbas, 2011; Maleki et al., 2013). Currently, more than 95% of the world biodiesel is produced from edible vegetable oils (Gui et al., 2008) such as rapeseed (Yoo et al., 2010; Tang et al., 2011), soybean (Cao et al., 2005; Likozar and Levec., 2014), palm (Hammed et al., 2009; Likozar and Levec., 2014) and sunflower oils (Yin et al., 2012; Likozar and Levec., 2014). Biodiesel production in a large scale raises great concerns due to food–fuel competition in the long term (Chhetri et al., 2008; Refaat et al., 2009). Therefore, using nonedible vegetable oils like Jatropha curcas (Kumar Tiwari et al.,2007; Ganapathy et al., 2009) and castor oil (Meneghetti et al., 2006) not only can skip the food–fuel concerns, but also decreases the final biodiesel cost which is rigorously affected by the feedstock type (Phan AN and Phan TM, 2008). The castor biodiesel has very interesting properties (very low cloud and pour points) that show that this fuel is very suitable for using in extreme winter temperatures (Beltrão, 2005). Castor oil and its derivative castor biodiesel is indispensable for preventing fuels and lubricants utilized in aircraft and space rockets from freezing at extremely low temperatures. Raw castor oils' major market is beginning to open in the energy field, with the growth of biodiesel. Each hectare of castor oil bean plants planted in arid and semiarid regions produces 350-650 kg of oil, which in turn produces 280-520 kg of biodiesel per hectare. Besides, castor bean leaves can serve as food for silkworms; the pulp for paper manufacture; the cake, coming after pressing the seeds, is used as organic compost possessing too, nematicidal effect; cultural waste can return 20 t/ha of biomass to the soil (Silva et al., 2008). From the industrial point of view castor oil is very important as the fundamental source of hydroxylated fatty acids (78-90%) for the oleochemical industry (Zanetti et al., 2013). Europe is the main world user of castor oil, presently the only commercial source of hydroxy fatty acids (HFA), consuming almost a quarter (150,000 tons per year) of the entire world production (Severino et al., 2012).

Market and exports of HFA-based products by European industries are growing rapidly and constantly. Nonetheless, castor crop has disappeared from Europe over the last twenty years, and European industries now import 100% of the castor oil used to extract HFA. What is worse, is that these imports come almost exclusively from just one country (India), with evident risks in terms of supply levels and price instability, that varied by up to 50% from one year to the next. The adaptability of castor to European pedo-climatic conditions has been widely demonstrated (Vannozzi et al., 1983; Arnaud, 1990; Baldanzi and Benventi, 1995; Laureti and Marras, 1995; Koutroubas et al., 1999); moreover, castor was extensively grown in Europe until the 80s', and wild types spontaneously grow in many areas of the Mediterranean region.

Therefore, developing castor in Europe in short term, as well as being strategically important, seems also extremely realistic, affordable and achievable (Alexopoulou et al., 2015).

1.1.2 Botanical Descriptions

Castor plant varies greatly in its growth habit, colour of foliage, stems, seed size colour and oil content so that varieties often bear little resemblance to one another. Castor may be large perennials often developing into small trees, others behave as short-lived dwarf annuals, commonly known as giant and dwarf types respectively. However, castor grows at an amazingly fast rate, if they are situated in full sun and provided with ample fertilizer and water. Giant types have a large, well-developed tap-root which can reach several feet in length and has substantial laterals and secondary roots. Root systems of dwarf types reflect the variety or cultural system and show less apparent tap-root (Weiss, 1983). The well-developed root system allows the plant to take maximum advantage of soil moisture, a major factor in the plants resistance to drought. Root system shows a strong correlation to yield because it allows the crop to tape necessary nutrient and water for proper accumulation of biomass.

Planting castor in a soft and loose soil is an advantage for proper development of root which will in turn contribute to better yield.

Castor stem is round, glabrous, frequently glaucous and it may reach 4–5 m in height. The stem may present different colours from green to red or purple. It is multi-branched, primary branches giving rise to secondary branches, a sequence that continues over the life of the plant. There are well-developed nodes, from each of which a leaf arises. The node at which the first racemes appear is an important agronomic characteristic, since it is associated with quick maturity.

In dwarf-internodes hybrids, it usually occurs after the sixth to twelfth node, but in segregating population can vary from six to forty-five (Weiss,1983). The leaves are large and about 15 to 45 centimetres long, with long petiole. The leaves are palmate with five to eleven lobes and prominent veins on the under surface. Leaves are alternate, except for two opposite leaves at the node immediately above cotyledons. The leaf colour varies from light green to dark red depending on the level of anthocyanin pigmentation present (Weiss, 2000). In some varieties, the leaves start – off as dark reddish purple or bronze when young but gradually changing to a dark green, sometimes with a reddish tinge as they mature. The leaves of some others are green practically from the start, whereas in others a pigment masks the green colour of all the Chlorophyll – bearing parts. Growth and expansion of castor leaves do not appear to be checked by prolonged sunlight provided there is ample moisture for transpiration. It is when a water deficit has been built up that leaf growth and expansion are affected. The reduction in leaf growth and expansion, and drastically falling of leaves during dry season, resulting in low surface area for photosynthetic activity, are the causes of reduction in yield observed during the season. The leaf diseases cause by some bacteria and fungi can also affect the yield (Weiss, 2000).

Castor is naturally a cross-pollinated plant and wind is the major agent of pollination. The castor flowers are borne on inflorescences, which forms a pyramidal raceme also known as spikes, terminally on main and lateral branches. The flowers may be monoecious (male and female), with male flowers on the lower portion of the raceme and female on the upper, may be pistillate (female only) or interspersed (arranged intersperse) along the length of the raceme. In normal monoecious varieties, the percentage of pistillate flowers along the raceme axis is usually the highest on the first raceme, with a decreasing percentage on subsequently developed racemes. With the decrease in pistillate flowers, there is a proportional increase in the number of staminate flowers (Zimmerman et al. 1966). This within plant variation is generally associated with the seasons. Female tendency is highest in spring and early summer; male tendency is highest in mid and late summer.

Temperature is probably the main environmental component affecting sex. Moderate temperatures promote female flowers while high temperature promote male flowers. However, age of plant and nutrition can also influence sex expression. Femaleness is strongest in young plants with a high level of nutrition. Maleness is strongest in old plants with a low level of nutrition (Shifriss, 1956). The inflorescence can reach a length of 100 cm, but since there is wide variation in distance between the flowers, ratio of male to female flowers and number of fertile female flowers, the yield is not necessary correlated with the length. In most castor varieties, female flowers open before the male while in others male open first (NCRI, 2013). The male flowers shed most of the viable pollens between 1 to 2 days after opening. The pollens normally shed from 2-3 hours before sunrise until late afternoon, and there is frequently a peak at midmorning. The pollen is shed readily between 26-29 °C with a relative humidity of 60 percent. The stigma can remain receptive for period of 5 to 10 days after opening. Days between the opening of female flowers and that of male may varies from 3 to 7 days depending on genotypes (Christopher, 1996). The fruit is a three-lobed, green or red capsule with a soft, spiny exterior. The castor fruit is usually a schizocarp, typical regma; a capsular fruit with three cells each of which splits open at maturity into separate parts and then breaks away explosively, shattering the seeds. The capsule contains three seeds which may be elongated, oval or square in shape. The seeds have a shining, marble-gray and brown, thick, leathery outer coat, within which is a thin, dark colored, brittle coat.

A large, distinct, leafy embryo lies in the middle of a dense, oily tissue (endosperm). The seed has a tiny and brittle testa (seed coat) enclosing a white kernel. The seeds may be coloured white, dark brownish-red, brown, dark chocolate, red or black but usually several colours occur as very attractive mottle on the testa. The seeds vary greatly in size, from a few millimeters to nearly 250 mm long and in breadth from 5 to 16 mm.100 seeds varies in weight from 10 to 100 g. The variation is not only among varieties but from different racemes. In general, the seed weight increases as the total number seeds produced per plant decreases. In some varieties, castor seeds may have a dormancy period of several months while freshly harvested seeds of some can germinate without special treatment.

However, large seeded castors often germinate earlier compared with tiny seed. The dormancy in some castor can be broken by soaking for 24hr in water or removing the caruncle and pierce the testa at the site. Germination is epigeal with the cotyledons coming out above the soil and expands as green leaves. The period from seedling emergence to capsules' maturity varies with genotypes. On average, it varies from 140-160 days. (NCRI, 2014).

It is a plant with C3 photosynthetic metabolism, which under high temperature conditions and relative humidity presents high photosynthetic rates but under low relative humidity conditions it is reduced drastically as a consequence of the stomatal closure (Dai et al., 1992).

1.1.3 Ecology

Castor is a hardy crop which survives in a wide range of ecology. Basically castor grows throughout the warm-temperate and tropical regions, it flourishes under varieties of climate conditions that its range cannot easily be defined. It growns almost anywhere land is available. Castor is basically a long-day plant, but is adaptable with fewer yields to a wide range of photoperiod.

However, castor flowers normally on both a short 12 hour and a long 18 hour day, but at 9 hours growth and development were severely retarded (Weiss, 1983). Castor grows in all kinds of soils but prefers a well drained moisture retentive soil like sandy loam. It grows well in on a rich soil and tolerates not less than daytime temperatures of 20 (Gana et al., 2013). Castor tolerates pH of 4.5 to 8.3 and annual temperature of 7 to 27.8 °C and annual precipitation of 200 to 4290 mm (Falasca et al., 2012). Although castor can be successfully cultivated in areas of marginal agronomic potential, production is nonetheless sensitive to extreme climatic variations, particularly with regard to rainfall distribution. Cultivated castor requires fertile, well aerated soils with a pH of 6 -7.3 and rainfall of 600-700 mm for optimum yield. Seed yield varies between variety, genotype, and provenance. Averages of 7650, 5940, and 3560 kg ha^{-1} are obtained for Tunisian, Brazilian, and Italian genotypes respectively (Anastasi et al., 2015). Insufficient nitrogen results in reduced seed yields. Excessive nitrogen results in extensive and heavy vegetative growth with nonsignificant increase in yields. The amount of nitrogen requirement depends on the soil organic matter content (Copani el al., 1995). Basically, castor requires the same amount of nutrients as other low-demand field crops (Gana et al., 2013).

1.1.4 Biotic Stresses

Among the major production constraints for the profitable production of this crop is the vulnerability of several castor varieties and hybrids to insect pests (Lakshminarayana and Raoof, 2005). The major pests on castor include the semilooper (*Achaea janata* L.), capsule borer (*Conogethes puncitiferalis*), *Spodoptera litura* Fabricius, red hairy caterpillar (*Amsacta albistriga* Walker), jassids (*Empoasca flavescens* Fabricius) and the white fly (*Trialeurodes ricini* Misra). Larvae of *A. janata* and *S. litura* (Noctuidae: Lepidoptera) are voracious foliage feeders which totally defoliate the plants. For effective management of the defoliators, mechanical control of *S. litura* in its gregarious stage and hand picking of older *A. janata* larvae are suggested (Lakshminarayana and Raoof, 2005).

A peak level of infestation of *A. janata* causes excessive defoliation affecting photosynthesis. Later, the larvae eat away the tender capsules of primary and secondary spikes. It is estimated that castor yields are reduced by 30–50% due to *A. janata alone*. Incidence of *C. punctiferalis* is commonly noticed in the later stage of crop growth, especially secondary and tertiary spike orders.

Larvae web the tender capsules, bore into them, and eat away the kernel. The borer attacks various plant parts such as the shoots, inflorescences, and capsules, causing considerable yield losses (Singh et al. 1992). Intercropping is an important cultural practice in pest management and is based on the principle of reducing insect pests by increasing the diversity of an ecosystem (Risch, 2005).

Castor is affected by several diseases; however, only a few are regarded to be of economic importance. The major diseases affecting castor are wilt-*Fusarium oxysporum* f. sp. ricini, root rot, stem rot & charcoal rot *Macrophomina phaseolina*, seedling blight *Phytophthora parasitica*, capsule rot *Cladosporium oxysporum*, fruit rot & Gray rot-*Botrytis ricini*, rust-*Melamspora ricini*, powdery mildew-*Leveillula taurica*, phyllosticta leaf spot-*Phyllosticta bosensis*, angular leaf spot-Botrytis sp., damping off *Phythium aphanidermatum* (Rangaswamy and Mahadevan, 2005). These diseases can reduce yield, production and germination up to 30-50% (Kumar et al., 2007). Wilt caused by *F. oxysporum* f. sp. *ricini* is the most important soil and seed borne disease of castor causing significant yield losses. Fusarium wilt in castor was first reported in 1937 from Brazil and in India from Udaipur, Rajasthan in 1974 (Nanda and Prasad, 1974). In India, the disease is observed in all the castor growing regions of the country. Fusarium wilt generally appears in patches and at all growth stages of the crop. Several others diseases can sporadically cause severe outbreaks depending on genotype and climatic conditions, such as the leaf spots caused by the fungus *Alternaria ricini* (Yoshii) Hansf. and *Cercospora ricinella* Saccardo and Berlese and the bacteria *Xanthomonas axonopodis* pv. ricini Hasse. Among these, *A. ricini* deserves more attention because it is a seedborne fungus that can also cause seedling blight and pod rot with seed yield losses reaching 70% (Holliday, 1980).

Castor belongs to the monotypic genus *Ricinus;* success in castor breeding with yield stability, has subsequently been limited by a lack of exploitable genetic variability for resistance to these insect pests.

With the development of biotechnology, it has become possible to create varieties through introducing exogenous or endogenous genes into the castor genome to express exogenous genes directly or regulate the expression of endogenous gene. In recent years, development of transgenic castor through exploitation of biotechnological tools is regarded as one of the promising approaches for incorporation of desirable traits for which sources are rather limited in castor germplasm. Researches on genetic transformation on disease resistance, insect resistance and low toxicity protein content has been conducted in castor through pollen tube pass way, *Agrobacterium tumefaciens* mediated transformation, particle bombardment and other methods, transgenic castor bean has been achieved successively (Lu et al., 2018).

1.1.5 Abiotic Stresses

Tolerance to environmental stresses, particularly to drought stress, is one of the strengths of castor as a crop. Because castor oil is an only-industrial product, there is a possibility that the competition for land with food crops moves castor production into marginal soils.

In that scenario, tolerance to abiotic stresses would be particularly important. Castor plants are more sensitive to water stress in the early stages of growth. At the cellular level, water stress reduced callus initiation, nitrate reductase activity, and chlorophyll content (Manjula et al., 2003a, 2003b). Drought stress increases cuticular wax load (Lakshmamma et al., 2009b) and abscisic acid concentration in the phloem sap (Zhong et al., 1996). Osmotic adjustment is an important mechanism for drought tolerance in castor. The seed yield of genotypes with higher osmotic adjustment was 53% greater compared to genotypes with low osmotic adjustment (Babita et al., 2010). Under limited water supply, castor plants maintained efficient stomatal control while keeping a high level of net CO_2 fixation. Water loss by transpiration was minimized by an early stomatal closure (Sausen and Rosa, 2010). It has been observed that under drought stress the photosynthetic apparatus of castor plants was preserved and that photosynthetic limitations were mostly due to diffusive resistance (Sausen and Rosa, 2010). Castor plants were able to partially recover photosynthetic functions while experiencing stress due to severe drought. When the stress was removed, the plants completely recovered their normal photosynthetic function within 24 h. However, castor showed high levels of sensitivity to limited light (Funk and Zachary, 2010).

Growth and production of castor were also inhibited by high salinity (Na) in the either the irrigation water or in the soil (Silva et al., 2008). Plants were most sensitive in the early stages of development (Pinheiro et al., 2008). Increasing salinity delayed and reduced total emergence of castor seed, but the genotype CSRN 367 was notably less sensitive to the effects of salinity than BRS Paraguaçu (Silva et al., 2005). The threshold of Na salinity for castor emergence and growth is 7.1 dS m^{-1} while the emergence rate was delayed by 9 d and was 50% lower at a salinity of 13.6 dS m^{-1}. Sixty percent of the seedlings did not survive when subjected to the same salinity level for 11 d (Zhou et al., 2010). Increasing levels of Na salinity apparently damaged the photosynthetic apparatus and induced proline accumulation in castor plants (Li et al., 2010). Castor is extremely sensitive to soil hypoxia. Within 2 to 6 hafter subjecting castor plants to soil flooding, Else et al. (2001) observed a reduction in stomatal conductance, transpiration, CO_2 uptake, leaf elongation, root hydraulic conductance, and production of abscisic acid from flooded roots. Castor plants subjected to continuous flooding were permanently damaged after 3 d and died after 4 d (Severino et al., 2005). Leaves of plants subjected to hypoxic condition had increased β-amylase activity; increased concentration of starch, protein, and soluble sugars; and reduced activity of nitrate reductase (Beltrão et al., 2003, 2006). Baldwin and Cossar (2009) also observed reduction in seed yield on castor fields subjected to flooding. Soil acidity can also impair castor growth. The growth of castor plants was reduced in a soil with 6.5 mmolc cm^{-3} of Al, but the addition of wood ash or bovine manure reduced the effect of acidity (Lima et al., 2009b).

Increased exchangeable Al content was also less harmful to the growth of castor plants when the soil had a high content of organic matter (Lima et al., 2007a). Temperature in the root zone can influence leaf growth, water status, and carbohydrate transport in castor plants. When air temperature was kept at 22°C for 34 d, plants with root-zone temperature at 20°C had 881 cm^2 of leaf area, while plants kept at 10°C had only 288 cm^2 of leaves. The shoot and root biomass production was three times smaller in the plants subjected to cold soil temperatures. When temperatures increased in the root-zone plants resumed normal leaf growth within a few days (Poire et al., 2010).

1.1.6 Development of Commercial Cultivars

The development of new castor cultivars would be enhanced by improved knowledge on the genetics and molecular biology of this species. Because castor is a cross-pollinated crop that has limited inbreeding depression, it is often treated as a self-pollinated crop in breeding programs (Moshkin, 1986; Lavanya and Chandramohan, 2003). Detailed descriptions of methods for castor breeding can be found in Kulkarni and Ramanamurthy (1977), Moshkin (1986), Lavanya et al. (2006), Auld et al. (2009), and Lavanya and Solanki (2010). Mass selection in castor has been effective for selection of traits with high heritabilities. This technique works best with selffertilization of selected plants to prevent cross pollination and controlled selection techniques to reduce environmental variation (Auld et al., 2009). Mass selection with self-pollination was the most effective method for increasing the frequency of pistillate castor plants of the type NES (NES type: the plant has the recessive gene (ff) that allows it to start as female, but the presence of environmentally sensitive genes triggers a sexual reversion when temperature is higher than 31 °C) (Bertozzo et al., 2011). Mass selection with intercrossing in isolated plots was used to develop Nila Bicentenaria, adapted to the Andean Region of Colombia (1500–2200 m above sea level) (Navas, unpublished data, 2012). The approach was also used to purify three cultivars highly variable for days to flowering and number of capsules per raceme (Reddy et al., 1999). Breeding to increase the harvest index (HI) is a promising avenue for improvement in castor oil yield. Castor seed HI can be as low as 0.1, while in many other cultivated crops it ranges from 0.4 to 0.6. The HI can be increased by selecting for reduced structural components such as plant height, stem diameter, petiole length, capsule spines density, capsule hulls and seed coat thickness, and caruncle weight. Seed oil content could also be enhanced by reducing the protein and carbohydrate content in the extensive endosperm of castor (Rana et al., 2006).

1.1.7 Agricultural Practices

In most of the regions of castor production, seed yield can be rapidly increased with the use of improved agronomical practices.

The main technologies include selection of the appropriate cultivar combined with use of good quality seed, appropriate planting date, irrigation, soil fertilization, management of weed, pest, and diseases, optimized plant population, mechanical harvesting, and postharvest management. Optimizing planting population is an inexpensive practice that can significantly increase castor seed yield.

However, the optimum plant population varies influenced by the genotype, environmental conditions, and agricultural practices.

Because environmental conditions are not constant, there is not an individual plant density that can be broadly recommended for castor. Instead, researchers should focus on determining a range of plant populations targeting the best yield across years and environmental conditions.

As an example of environment influence, plant populations recommended for the cultivar BRS Nordestina (tall type) in different locations were 5000 plant ha^{-1} (Severino et al., 2006d), 4200 plant ha^{-1} (Severino et al., 2006a), and 12500 plant ha^{-1} (Carvalho et al., 2010a). In the cultivar Guarani (tall type), plant populations ranging from 10000 to 22222 plant ha^{-1} did not affect seed yield (Bizinoto et al., 2010). In the cultivar FCA PB (short type), the seed yield was 22% higher (4100 kg ha^{-1}) using an optimized plant population in the range of 55000 to 70000 plant ha^{-1} and row spacing of 0.45 to 0.75 m (Soratto et al., 2011). Planting date can also influence castor seed yield. Castor seed yield varied from 89 to1954 kg ha^{-1} among four locations and six planting dates in the States of Mississippi and Tennessee (Baldwin and Cossar, 2009). The greatest yields were obtained with early spring planting. Planting date also impacted the occurrence of pests and diseases in the State of Rio Grande do Sul, Brazil (Zuchi et al., 2010a). Preliminary field experiments carried out in southern Sicily and in Tunisia have shown the possibility of exploiting the perennial habit of this species (Sortino et al., 2009; Sortino et al., 2010a; Sortino et al., 2010b). Studies on seed yield components suggested that the increase of one yield component does not always result in a proportional increase in seed yield because other components compensate (Fanan et al., 2009; Zuchi et al., 2010c). Studies vary regarding the impact of first, second, and third racemes on final seed yield because of the genotype and environmental factors (Fanan et al., 2009; Neto et al., 2009; Zuchi et al., 2010b, 2010c; Vallejos et al., 2011). In castor plants subjected to repeated defoliations, the seed yield component with the widest adaptation was the number of racemes (Severino et al., 2010). Seed yield depends on the number of racemes per plant, the number of capsules per raceme and the thousand seed weight. Under natural conditions, the castor plant has many racemes, depending on the number of branches that develop progressively over the life of the plant. This is undesirable characteristic for commercial production since the high number of racemes results in an extended maturity period, thus making mechanical harvesting uneconomical. Low branched plants with one to three racemes resistant to shattering are desirable in modern castor varieties. The number of capsules per raceme depends on the number of female flowers on the raceme. The proportion of male and female flowers on each raceme varies and can be influenced by the environment. (Weiss, 1983). The proportion of female flowers is reduced by temperatures above 30°C, increasing plant age, higher raceme position, inadequate mineral nutrition, and sudden changes in temperature (Lakshmamma et al., 2002; Lavanya, 2002; Neeraja et al., 2010). Consequently, the proportion of female flowers ratio influence on seed yield needs to be further investigated.

Recent research indicates that castor seed yield does not appear to be sinklimited but source-limited (Severino, unpublished data, 2012).

Therefore, increasing the proportion of female flowers would not necessarily increase seed yield if assimilates and nutrients were in fact the factor limiting seed production. India has been successful in the development of high yielding hybrids, but further seed yield increases can be achieved. In the State of Tamil Nadu (India), a 72% seed yield increase was obtained with adoption of hybrids, better weed management, and pest control (Manickan et al., 2009b). The average seed yields in India range from 1864 kg ha^{-1} in the State of

Gujarat to 371 kg ha^{-1} in the State of Andra Pradesh, where the crop has been predominantly grown without irrigation on marginal soils (Basappa, 2007). In Brazil, seed yields have averaged 667 kg ha^{-1} over the last 10 year (CONAB, 2011). The State of Parana has the highest average seed yield in the country (1600 kg ha^{-1}) due to better soil fertility and agronomical practices (Silva et al., 2009). The use of high yielding castor genotypes has been very limited because of not mechanized production, limited use of fertilization, and the lack of good agronomic practices (Santos et al., 2001). Hence, 90% of the castor is grown in Brazil on small farms of <5 ha, with the use of low quality seed, poor management, insufficient technical assistance, primitive agronomical practices, and scarcity of credit (Queiroga and Santos, 2008; Silva, 2009). Propagation of castor using seedlings started in plastic bags or root plugs has been studied as an alternative for regions with short-growing seasons (Lima et al., 2006a, 2006b, 2007b). While castor production is feasible under such conditions, it is limited by the fragile castor roots, malformed root systems, and the high costs of production. In a no-till system, the highest castor seed yield was obtained when the previous crop was a mix of sunhemp (*Crotalaria juncea* L.) and pearl millet (*Pennisetum glaucum* L.). It seems that the high N content of sunhemp and the high biomass volume of pearl millet created an ideal environment for castor production (Silva et al., 2010a).

However, chopping the mulch before planting castor caused a quick decomposition of the covering biomass and reduced castor seed yield (Silva et al., 2010a). Castor plants can lose leaves because of pests, diseases, wind, hail, machinery traffic, and inappropriate use of herbicides and defoliants.

A castor plant can recover from a drastic defoliation; however, damage to leaves always causes a reduction on seed yield. For each 1 m^2 of lost leaf area, production is reduced by 37.8 g of seed and 24.4 g of oil (Lakshmamma et al., 2009a; Lakshmi et al., 2010; Severino et al., 2010). Two systems for castor production differing in the degree of mechanization were compared for energy returned over the invested energy (EROI) (Silva et al., 2010b). The system with less intensive mechanization consumed less energy and had a higher relative energy gain. This low-mechanization system produced less net energy and required more cropping area to produce a similar amount of net energy. The production of 1000 GJ of net energy required an area of 66.2 ha with low-mechanization compared to 37.5 ha with highly mechanized crop production (Silva et al., 2010b).

The highly mechanized cultivation also used more nonrenewable resources, including limestone, fuel, and fertilizer. The EROIs of both production systems showed that castor was not very efficient as an energy source. This area needs further study if castor is to be considered as a viable biofuel feedstock. Although mechanized harvest is a priority for expanded castor production, there is no study on mechanization of castor production besides breeding for appropriate plant architecture.

Some topics for future research on mechanization include the optimization of harvesting machinery, development of options for mechanized harvest of small fields, managing fruit maturation and dehisensce to optimize harvest, influence of harvest on seed quality, postharvest seed quality, necessity of dedicated castor machinery, and the cost of mechanical harvesting.

1.1.8 Irrigation

Castor plants have high levels of drought tolerance, but seed yields are reduced under limited water supply. Castor can produce a low seed yield under low water availability where some species would not make a crop; however, only with an adequate water supply seed yield can be optimized, particularly with genotypes with high yield potential. Water requirement for castor varies from 466 mm to 1178 mm (Patel et al., 1998) depending on soil type and location.

Under an optimized irrigation level, a seed yield of 3780 kg ha^{-1} was obtained in India with the hybrid GCH-5 (Raj et al., 2010). In Greece, the seed yield of 1080 kg ha^{-1} observed on the hybrid Pronto with 147 mm of rain was increased to 4040 kg ha^{-1} due to the addition of 363 mm of water through irrigation (Koutroubas et al., 1999). A castor field produced 1774 kg ha^{-1} of seeds without irrigation, 2199 kg ha^{-1} with a supplementary irrigation at the end of the growing season, and 4252 kg ha^{-1} with supplementary irrigation before and after the rainy season (Souza et al., 2007).

Laureti and Marras (1995) found a linear increase in yield with increased water application until 100% of evapotranspiration. The positive response of yield to irrigation was associated with an increase in seed weight. The yield increase in response to irrigation was associated with more capsules per plant on the first and other racemes and with more seeds per capsule. Irrigation was found necessary for castor production since seed and oil yields obtained with irrigation were much higher compared to the over years mean yields of rainfed plants (Koutroubas et al., 2000).

The castor crop is one of the largest crops not destined for consumption used in arid and semiarid zones, by its easy cultivation, its drought and salinity tolerance and besides being promising alternative for biofuel production (Beltrão et al., 2010; Nahar, 2013). In the semiarid region, rainfall distribution is irregular, which may cause inability of agricultural lands. So, the use of irrigation is indispensable to increase agricultural productivity (Frizzone et al., 1994). However, under these environmental conditions, as it has been demonstrated for other macrothermal species such as kenaf and sunflower, early sowings could be advantageous also for castor, as they allow the root system to exploit the water stored in the soil during the winter period, reducing irrigation requirements (Patanè et al., 1995; Anastasi et al., 2000; Patanè and Sortino, 2010,). The growth and development of castor plants are affected by high concentrations of salinity in both irrigation water and soil. The limit that has been reported for emergence and growth is 7.1 dS m^{-1}, with which emergence is delayed by 9 days (Severino et al., 2012). The salinity threshold for castor seed germination was found to be 7.1 dS m^{-1} (caused by NaCl) because the germination was less than 75% and less than 70% of the seedlings survived with a salinity higher than that (Zhou et al., 2010). Reduced and slower germination of castor seed due to salinity is commonly observed (Silva et al., 2008; Severino et al., 2012b, Nobre et al., 2013). The tolerance to salinity was different among genotypes (Silva et al., 2008). While in tropical regions castor is a perennial species, in more temperate areas such those of Mediterranean basin it is normally grown as an annual crop (Severino et al., 2012).

1.1.9 Fertilization and Plant Nutrition

A castor field yielding 2000 kg ha^{-1} of seed will remove from the soil 80 kg ha^{-1} of N, 18 kg ha^{-1} of P_2O_5, 32 kg ha^{-1} of K_2O, 13 kg ha^{-1} of CaO, and 10 kg ha^{-1} of MgO (Filho and Freire, 1958). If husks are not returned to the soil after crushing, this removal is even higher (Severino et al., 2006b). In Brazil, maximum seed yields were obtained with an application of 59 kg ha^{-1} of N, while no effect of additions of P, K, or minor nutrients was observed (Severino et al., 2006c). Mineral fertilizers were more effective than bovine manure for supplying N due to slow mineralization of organic materials in a semiarid environment (Severino et al., 2006b). Silva et al. (2007) observed increased castor seed yield when N-rich fertilizers were added up to 80 kg ha^{-1}. The best time for N application for two hybrids cultivated as second crop in a non till system varied according to the genotype and environmental conditions (Moro et al., 2011). The application of P increased castor seed yield in a soil with high levels of K and organic matter (Pacheco et al., 2008), and in an acidic soil in the State of Alagoas, Brazil (Silva et al., 2012). When water was a more limiting factor than soil nutrients, castor seed yield showed only a limited response to N, P, and K fertilizers (Neto et al., 2009).

When castor was cultivated in rotation after sugarcane, seed yield was increased by 90% when the soil was fertilized with 10 t ha–1 of sewage sludge (Chiaradia et al., 2009). In India, seed yield increases in irrigated castor hybrid GCH-4 were observed after fertilization with N and K (Hadvani et al., 2010). In semiarid regions of India where deficiency of S, B, and Zn are widespread, the fertilization with these three nutrients increased the seed yield of rain-fed castor in commercial fields from 757 to 1043 kg ha–1 over 3 yr of study. When N and P were also applied, seed yields were increased an additional 15% (Sahrawat et al., 2010). In an acidic (pH 4.2), dystrophic, Red Latosoil, the addition of lime increased castor seed yield, but addition of Zn had no effect on plant growth or production (Leles et al., 2010). Castor seed immersed in solutions containing the micronutrients Fe, Zn, and Mo before germination exhibited increased germination rates and seedling dry mass but a reduced germination rate when treated with B (de Oliveira et al., 2010).

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1.2 Aim of the thesis

The present research project aims to contribute to create a biorefinery based on Castor in semi-arid Mediterranean environment. Specific objectives were:

- To identify the requirements in water and nitrogen and to study the morphologic, physiologic and productive response of castor bean to water and nitrogen stress.

- To improve the energy conversion process (biomethane production).

In order to reach the main goal, the project was splitted in two main research lines:

1) The optimization of the cultivation techniques through the evaluation of different agronomical inputs.

In this line, a local variety of castor was evaluated in relation to different agronomical practices.

The response of Castor to different levels of irrigation and fertilization was studied in terms of seed and oil yield, phenological stages duration and physiological parameters (photosynthesis, transpiration, stomatal conductance, fluorescence). The local variety was also compared with four dwarf annual hybrids evaluating different sowing date to verify the best period of cultivation.

2) The improvement of energy conversion process through the biomethane production from residual biomass.

With this aim, in a perspective of circular economy and reuse of the waste, Castor oil was chemically extracted to determine oil content and oil yield, whereas capsule husks, an agricultural lignocellulosic residue obtained from the oil extraction process, were used as substrate to evaluate the experimental biomethane production by BMP (Biochemical Methane Potential) test through anaerobic digestion process. A step of pretreatment was needed in order to alter the biomass structure and partially degrade lignin to enhance biomass digestibility. We adopted a biological pretreatment using white-rot fungi (*Pleurotus ostreatus* and *Irpex lacteus*).

1.3 Thesis outline

The thesis is organized in six main chapters. Chapter 1 is a general introduction that presents the state of art of Castor cultivation, its main agronomical practices and industrial applications.

The results of field and laboratory experiments are reported in Chapter 2, 3, 4, 5.

Chapter 2 presents the evaluation of seed and oil yield of a local variety of Castor by changing irrigation levels and nitrogen fertilization doses under the semiarid Mediterranean area.

Chapter 3 reports the results of field experiment conducted to compare the behavior of a local variety of Castor with four dwarf annual hybrids in response to different sowing dates in order to verify the best period of cultivation.

Chapter 4 investigates the optimization of the cultivation techniques of a Castor local type selected by Di3A from Tunisian genotype, keeping the crop over a two- year period, through the evaluation of different agronomical inputs in order to increase seed and oil yield.

Chapter 5 investigates the effects of different nitrogen fertilization levels (0 and 120 kg ha^{-1}) on seed and oil yield and some agronomic traits of Castor. Moreover, in a biorefinery perspective, residual capsule husks obtained from seed processing, were analyzed in term of fibrous composition and biomethane production carrying out a biological pretreatment using two white rot fungi (*Pleurotus* *ostreatus* and *Irpex lacteus*).

The general conclusions of the results obtained are presented in Chapter 6.

2 Castor Oil As An Innovative Raw Material For An Energy Supply Chain In Semi-Arid Environments

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CASTOR OIL AS AN INNOVATIVE RAW MATERIAL FOR AN ENERGY SUPPLY CHAIN IN SEMI-ARID ENVIRONMENTS

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ABSTRACT: Increasing energy demand and fossil fuel prices together with environmental concerns have motivated scientists to find alternative energy sources such as biofuels. Biofuels are renewable and biodegradable sources of energy with beneficial environmental impacts. Biodiesel is a mixture of mono alkyl esters of vegetable oils or animal fats. Currently, more than 95% of the world biodiesel is produced from edible vegetable oils through different transesterification systems. Castor oil is an excellent raw material in terms of price and quality, but especially this non-edible vegetable oil does not have any issues or compromise food security. Recently, the use of castor oil has attracted attention for producing and optimizing biodiesel production, due to its high content and the possibility to esterify with only methanol, which assures low production costs. Additionally, the use of feedstocks as vegetable oils can contribute to decreasing greenhouses gases such as carbon dioxide, because these oils are obtained from crops which have previously captured this gas during their photosynthetic process. The innovative aspect of this study is the evaluation of the morphologic and productive response of castor bean to changing soil water and nitrogen fertilization under the semiarid Mediterranean climate.

KEYWORDS: *Ricinus communis* L., seed yield, oil yield, irrigation, nitrogen

2.1 Introduction

Castor (*Ricinus communis* L.) is a non-food, drought resistant, energy crop well known since antiquities, for the peculiar characteristics of its oil, being evidences of castor oil uses since Egyptian times (Scarpa and Guerci, 1982; Franz, 1988). Castor is a member of the Euphorbiaceae family that is found across all the tropical and semi-tropical regions of the world (Weiss, 2000). Castor was initially believed to have four centers of origin: (i) East Africa (Ethiopia), (ii) Northwest and Southwest Asia and Arabian Peninsula, (iii) India, and (iv) China. However, Ethiopia is considered to be the most probable site of origin because of the presence of high diversity (Moshkin, 1986; Anjani, 2012). It is cultivated in 1.5 million ha worldwide, mainly in India, China, Brazil, and Mozambique (Fernández-Martínez et al., 2012).

Castor is one of the largest crop not used for food consumption, very suited to arid and semiarid zones, by its easy cultivation, its drought and salinity tolerance and adaptation to several growing conditions (Lima et al., 2007; Babita et al., 2010, Beltrão et al., 2010; Patanè et al., 2019).

Castor seed contains between 40% and 60% oil. It is made up of the following fatty acids 91–95% ricinoleic acid, 4–5% linoleic acid, and 1–2% palmitic and stearic acids (Beltrão, 2005). The oil extracted from the castor seed has already a growing international market, assured by more than 700 uses, ranging from medicines and cosmetics to substituting petroleum in the manufacturing of plastics, lubricants and biodiesel (Azevedo et al., 1997; Freire, 2001).

Biodiesel are becoming big policy and big business as countries around the world are seeking to decrease petroleum dependence, reduce greenhouse gas (GHG) emissions in the transportation sector, and support agricultural interests (Berman et al., 2011). The adaptability of castor to European pedo-climatic conditions has been widely demonstrated (Vanozzi et al., 1983; Arnaud, 1990; Baldanzi and Benvenuti, 1995; Laureti and Marras, 1995; Koutroubas et al., 1999); moreover, castor was extensively grown in Europe until the 80s', and wild types spontaneously grow in many areas of the Mediterranean region, especially in the semiarid ones. Therefore, developing castor in Europe in short term, as well as being strategically important, seems also extremely realistic, affordable and achievable (Alexopoulou, 2015).

Preliminary field experiments carried out in southern Sicily and in Tunisia have shown the possibility of exploiting the perennial habit of this species (Sortino et al., 2010 a-b).

The present study investigates the optimization of cultivation techniques through the evaluation of the reduction of agronomical inputs in order to increase seed yield thereby reducing environmental burdens, by changing irrigation levels and nitrogen fertilization doses under the semiarid Mediterranean area.

2.2 Material and methods

2.2.1 Field trial description

Field experiments were conducted over the period 2020-2021 at the Experimental farm of the University of Catania, Italy (10 m a.s.l., 37°25' N lat., 15° 03' E long.) in a typical xerofluvent soil.

The soil of the experimental area was ploughed before sowing and fertilized with 70 kg/ha N (as ammonium nitrate). Two experimental factors were studied: irrigation and fertilization. Irrigation was applied as main plot and fertilization as sub plot. Four irrigation levels were employed: irrigation only at sowing (I0) and restitution of the 30 (I30), 60 (I60) and 100% (I100) of the evapotranspiration and three fertilization levels: 0 (N0), 60 (N60) and 120 (N120) kg N ha^{-1} (as ammonium sulfate).

A randomized block design with three replications was used, with fertilization levels randomly assigned within the main irrigation plot. Each plot measured 8 x 8.5 m and consisted of five rows. Sowing was carried out in June 2020. Castor seeds were sown at 3 to 4 cm depth and 1 m within row, 1.7 m apart (sowing density 0.58 plants per $1m^2$).

The irrigation volume was determined as the maximum available soil water content in a 0.6 m soil depth where root system is predominantly developed.

Irrigation was scheduled when the sum of daily maximum crop evapotranspiration (ETm) corresponded to the volume, subtracting rainfall events from the calculation. The daily ETm was calculated according to:

$$ETm = E0 \times Kp \times Kc$$

where ETm is the maximum daily evapotranspiration (mm); E0 is the evaporation of class-A pan (mm); Kp is the pan coefficient, equal to 0.80 in semi-arid environment. Crop coefficients were determined by previous observation (Kc): 0.4 from emergence to the 4 leaf stage; 0.7 from the 4 leaf stage to flowering; 1.2 from beginning of flowering to complete capsule development of the first raceme and 0.55 from first raceme to complete capsule senescence. The irrigation volume was calculated according to:

$$V = 0.66 \times (FC - WP) \times \phi \times D \times 10^3$$

where V = water amount (mm); 0.66 = readily available water not limiting for evapotranspiration; FC = soil water content at field capacity (27% of dry soil weight); WP = soil water content at wilting point (11% of dry soil weight); ϕ = bulk density (1.1 g cm^{-3}); and D = rooting depth (0.6 m).

2.2.2 Measurements and calculations

During the study, meteorological conditions and potential evapotranspiration (ET0) were continuously measured through a weather station connected to a data logger (Delta-T, WS-GP1 Compact) and a Class A evaporation pan (mm d^{-1}).

During the growing season, were be recorded the date of occurrence of the main phenological stages of crop (seedling emergence, flowering, brown full capsule, first brown raceme, seed physiological maturity) to evaluate the different response to the irrigation and fertilization levels.

The harvest was carried out starting from November 2020 according to the different flowering period.

At the harvest the number of raceme per plant and the number of capsules per raceme were measured. The first raceme and the other racemes were collected separately for seed yield. The seeds were separated from capsule residue to obtain clean seeds. A knife mill (GM200, Retsch) was used to crush seed into paste (cake) in preparation for oil extraction. The oil content was determined according to Randall method by the use of a solvent extractor SER 148 Velp Scientific in triplicate.

2.3 Results and discussions

Irrigation is the main factor influencing the phenological stages durations. Low levels of soil water availability induce a delay of anthesis and consequently seed ripening (Figure 1 and 2).

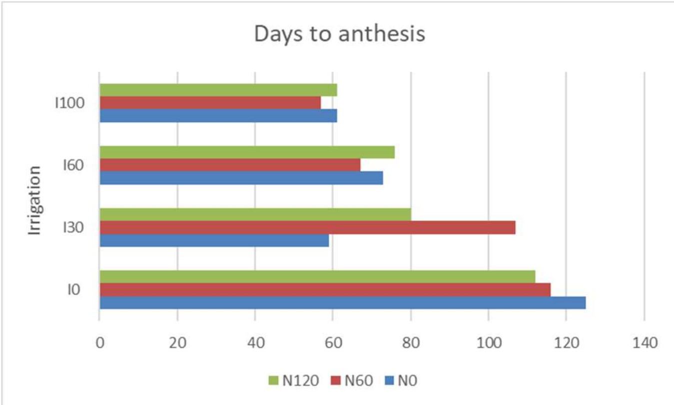

Figure 1. Phenological stage duration "sowing-flowering" (days) of castor under irrigation and fertilization levels.

The response of phenological stages duration to nitrogen availability is unclear in both investigated phenological stages. High level of soil nitrogen combined with low water availability induces a delay of seed ripening.

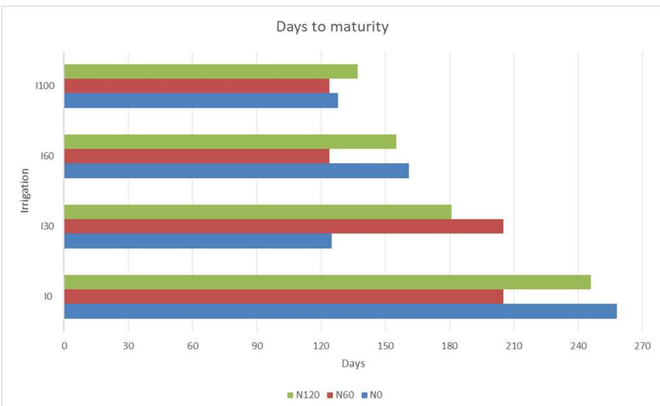

Figure 2. Phenological stage duration "sowing-seed maturation" (days) of castor under irrigation and fertilization levels.

Irrigation and fertilization influenced the number of secondary racemes per plant. High levels of water availability allowed for the development of secondary racemes. High soil nitrogen level improved the vegetative growth delaying the formation of secondary racemes (Figure 3).

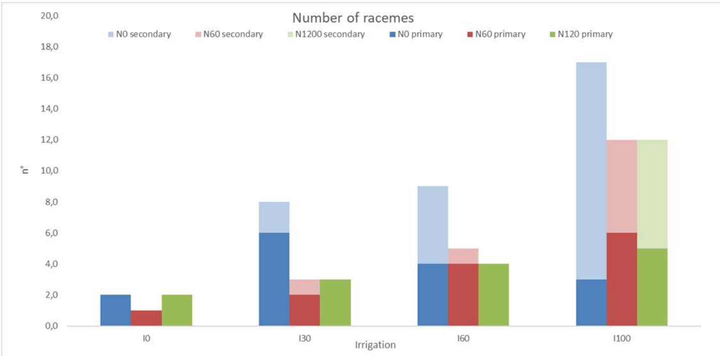

Figure 3. Raceme number of first and second order of castor under irrigation and fertilization levels.

Irrigation is also the main factor influencing seed yield. Higher soil water availability induced higher photosynthesis rate (data not shown) and consequently higher seed production (Figure 4). Fertilization had a positive effect on the seed yield from primary racemes, but induced a delay in the development of secondary racemes, causing a reduction of mean seed yield from secondary racemes.

Excessive nitrogen results in extensive and heavy vegetative growth with non-significant increase in yields, which, however, depends on the soil organic matter content and soil nitrogen availability (Gana et al., 2013).

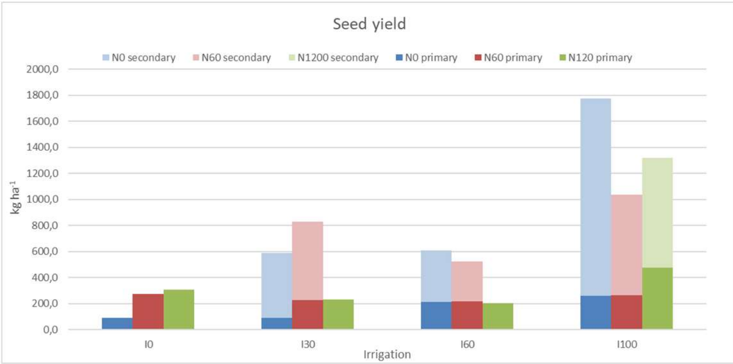

Figure 4. Seed yield (kg ha^{-1}) of first and second order raceme of castor under irrigation and fertilization levels.

Laureti and Marras (1995) found a linear increase in yield with increased water application until 100% of evapotranspiration. The positive response of yield to irrigation was associated with an increase in seed weight.

The yield increase in response to irrigation was associated with more capsules per plant on the first and other racemes. Irrigation was found necessary for castor production since seed and oil yields obtained with irrigation were much higher than rainfed plants (Koutroubas et al., 2000). Indeed, differently than the number of seeds per capsule, which has a high genetic heritability, the productivity of racemes is strongly influenced by the agronomic factors (Freire et al., 2007; Soratto et al., 2012).

Seed oil content increases with higher fertilization levels (Figure

5). Irrigation, on the other hand, increased seed oil content up to 60% ETm, and subsequently reduced at 100% ETm. Oil content is a complex quantitative trait that is networked with other storage and structural compounds in seed, and it is influenced by seed development and environmental conditions (Brummer et al., 1997; Abbadi and Leckband, 2011). Probably, the higher seed yield contributed to reduce the oil content in the seeds, however this needs further investigations.

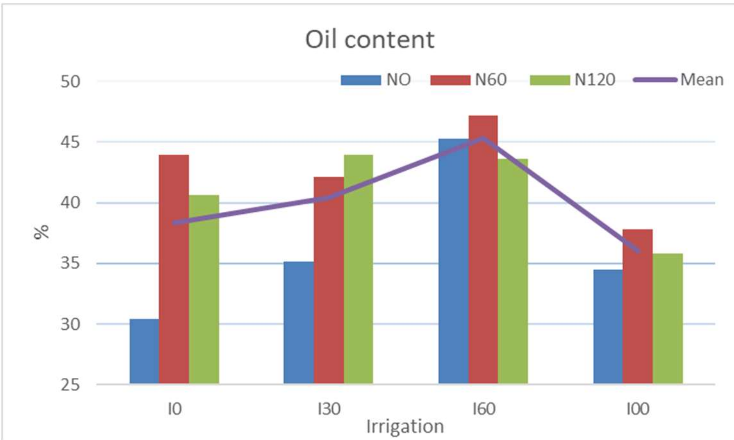

Figure 5. Oil content (%) of castor under irrigation and fertilization levels.

2.4 Conclusions

It is worth mentioning that present data refer to the establishment year and first harvest of a perennial castor type, and significant yield increases can be expected during the subsequent years.

Significant increases in seed yield were obtained with increased water supply. The yield increase in response to irrigation was associated with more racemes per plant, more capsules per plant on the first and other racemes and with higher seeds weight per capsule. On the other hand, the seed oil content seems to reduce under the highest soil water content.

The effect of nitrogen fertilization on yield component is less marked, mainly due the influence on physiological stages duration. High soil nitrogen content combined with low water availability is detrimental for castor productivity.

Castor plant has a high potential for use as a biorefining feedstock. The oil could be used for biodiesel production while other parts of the plant, including stem, seed cake, and leaves, which are among the lignocellulosic materials, could be used for advanced biogas and ethanol production.

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3 The Best Castor Hybrids Sowing Date In Order To Escape Adverse Weather During Start And Final Phases Of Growth Cycle

Based on: Calcagno S, Copani V, Castiglione R, Buscemi L, Piccitto A, Scordia D, Testa G, Cafaro V, Cosentino SL. The best castor Hybrids sowing date in order to escape adverse weather during start and final phases of growth cycle. In European Biomass Conference and Exhibition Proceedings; 2022; pp. 116-119. ISBN: 978-88-89407-22-6, DOI:10.5071/30thEUBCE2022-1AV.4.3.

THE BEST CASTOR HYBRIDS SOWING DATE IN ORDER TO ESCAPE ADVERSE WEATHER DURING START AND FINAL PHASES OF GROWTH CYCLE

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ABSTRACT: Belonging to the Euphorbiaceae family, Castor bean (*Ricinus communis* L.) is the most important non-edible industrial oilseed crop due to its high oil content that is around 40/55%. Castor is expected to be one of the most interesting oilseed crop to develop and study in the Mediterranean area in order to enhance the cultivation in degraded and marginal land.

Field experiment were conducted at the experimental farm of the University of Catania between the period of April and November 2021.

The aim was to study the behavior of a local variety of castor bean in comparison with four dwarf hybrids evaluate which sowing date was the best to cultivate castor for avoiding the low-winter temperature and verify the best period of cultivation. The flowering of the first

three sowing dates were recorded around the end of July due to temperature and photoperiodic effect.

The seed yield was higher for the dwarf hybrids, while the local variety had higher production for the primary racemes because of the shifting of the sowing dates which reduces the growing season.

KEYWORDS: *Ricinus communis* L., sowing dates, seed yield, phenological phases, Mediterranean environment.

3.1 Introduction

Castor bean (*Ricinus communis* L.), the only species of the genus Ricinus, is a plant belonging to the Euphorbiaceae or spurge family. Despite it is known as Castor bean, it does not belongs to the Fabaceae family and the seeds are not real beans or legumes. Native from tropical Africa, and mainly cultivated in India, Brazil, Mozambique and China (Chakrabarty et al., 2021), nowadays it is spread all over the world, compatibly with the climatic conditions. It has four known centres of origin (i) east Africa (Ethiopia), (ii) north-west and south-west Asia and Arabian Peninsula, (iii) India and (iv) China. It occurs as an herbaceous or arborescent plant, annual herb or perennial shrub, or small tree according to the climatic conditions of the region taken into consideration and on the cultivation techniques (Severino et al., 2012).

Castor ($2n = 2x = 20$) is a cross-pollinated diploid and an oilseed crop with a yield of around 40/55% (Kole and Rabinowicz, 2018).

On the contrary of other oilseed crops, Castor is characterized by a toxin glycoprotein present in high concentration in the seeds.

Several studies indicate that it was already used in ancient Egypt, 4000 BC, especially for its purgative properties (Dawson, 1929). Besides its laxative property, the uses and possibilities of Castor are different. Its oil and derivatives can be used in paints and varnishes, it is also a raw material for the production of sebacic acid used for different productions such as nylon and other resins, hydraulic fluids.

Even though the smell and taste are very unpleasant, it is widely used in the cosmetic industry without any problem mixed with other ingredients that mask this feature. Its oil is also used as insecticide, or as repellent for moles, gophers and vertebrate pests. (Ogunniyi, 2005).

The main use of Castor remains the production of fuels such as biodiesel. In fact, the growing and renewed interest in Castor worldwide is due to the need to obtain vegetable oils for the transformation into biodiesel, to replace fuels derived from fossil sources. In line with today's energy and agricultural policies, Castor bean is expected to be one of the most interesting crops to develop and cultivate in the Mediterranean environment. In the coming decade, its enhancement in marginal lands could be a valid alternative, not only because of the possibility of re-evaluate these abandoning and degraded lands but also to in order to achieve the goals settled up in the Agenda 2030 (ONU, 2015).

In this scenario, it is easy to understand the importance of studying the Mediterranean environment and the behaviour of local Castor varieties in order to make the most of its production possibilities and enhance the crop cultivation.

As already mentioned by Patanè et al., (2019) perennial plants are negatively impacted by low temperatures during the winter, in particular low soil temperatures delay the germination and the seedlings emergence of castor and extending the growing season allow a greater development of the plant by enhancing its productivity and allow the seed ripening stage to be reached earlier.

The spontaneous plant is a perennial shrub that can reach 3 meter height and produce ripe racemes progressively. This type of plant is not adapted to mechanization because of the presence at the same time of racemes with different age. Thus new hybrids more compact and more contemporary ripening of racemes were selected (Kaiima).

The present work has evaluated a reduction of the growing season by studying new annual hybrids which will grow testing the best sowing date in order to escape low temperature and verify the best period of cultivation.

3.2 Material and methods

3.2.1 Field trial description

The experiment was carried out at the Experimental farm of the University of Catania, Italy (10 m a.s.l., Catania (37°24'31"N; 15° 3'33"E) in a typical xerofluvent soil, during the period April-November 2021. The University of Catania is located in a warm Mediterranean climate (CSa), shown warm and dry summers and cool and wet winters (Peel et al., 2007).

The following factors were studied: genotypes of castor and sowing dates. The genotypes were five, four dwarf hybrids of Kaiima Company (C1012, C1008, C857, and C856) compared to a local selected population. The Kaiima genotypes are characterized by an early ripening cycle, suitable for annual mechanized harvesting.

The sowing dates were: S1= 01/04/21; S2= 30/04/21; S3= 01/06/21; S4= 08/07/21. In the experiment the dwarf hybrids has been identified as G1, G2, G3, and G4 while the local variety as GT.

The soil used during the experiment was ploughed before sowing. It was fertilized with 120 kg ha^{-1} of nitrogen distributed before the sowing and after it and 80 kg ha^{-1} of phosphorus distributed during sowing.

Castor seeds were sown to a depth of 3-4 cm and an interval within the row of about 0, 33 cm and between the rows 1 m. The plant density was of 3 plants per square meter.

The plants were irrigated with just one level of irrigation (100% of ETm) and it was scheduled when the sum of daily maximum crop evapotranspiration (ETm) corresponded to the volume, subtracting rainfall events from the calculation.

For each sowing period, the experimental design foresees five blocks in which the four dwarf genotypes and the local variety were randomly distributed. Sowing at the same time with the local variety made it possible to carry out a comparison study with the hybrids. The sowing was done manually and the field was irrigated before and after the sowing. Drip irrigation was carried out supplying 2820, 2112, 1816, 1615 m^3 of water in S1, S2, S3, and S4 respectively.

3.2.2 Measurements and calculations

During the growing season, phenological scoring was performed on each genotype for all the blocks, twice a week in order to mark the main phenological phases (E = seedling emergence, F1= flowering, brown full capsule, first brown raceme physiological maturity of the seed). The physiological maturity of the raceme was considered when the complete browning of capsules occurred.

The first harvest was carried out on 12/10/21 on primary and secondary racemes, while the second harvest was carried out on 23/11/21 on secondary and tertiary racemes left.

At the harvest, the number of main, secondary and tertiary racemes were measured. All racemes were collected separately in order to obtain individual yields by type of raceme collected. Then the seeds were separated from capsule residue to obtain clean seeds.

For the duration of the experiment, meteorological conditions and in particular, the temperature and rainfall data were measured through a weather station connected to a data logger (Delta-T, WS-GP1 Compact). The temperatures considered are the minimum and maximum temperatures measured during the duration of the experiment from April, the date of the first sowing, to November, the date of the last harvest (Table 2).

During the growing season of Castor, April to November considering the first sowing and the last harvest, the average temperatures were 26,8°C for the maximum and 16°C for the minimum and 21,3°C for the mean temperature (Figure 1).

Rainfall were more abundant during the period that went from September to November, with an average of 29.9 mm. The maximum of the rainfall was reached in the third decade of October with a maximum of 363 mm. The reference evapotranspiration (ET0) was higher during the summer season (June-August). The highest was concentrated in the third decade of July with a maximum of 69, 8 mm day^{-1}.

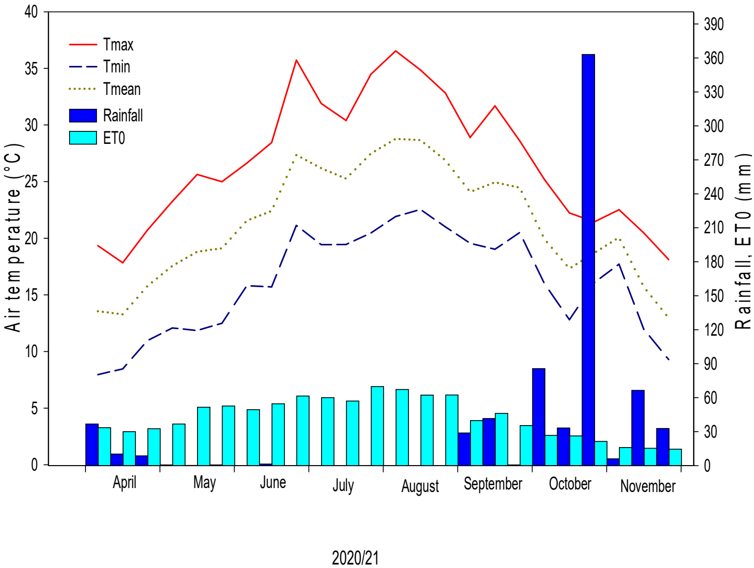

Figure 1. Meteorological parameters in spring and summer and autumn during the growing season at the Experimental farm of the University of Catania, Italy (37°25' N., 15°03' E., 10 m a.s.l.).

The scoring of the phenological phases has been performed during the experiment, for the production of the primary racemes in each sowing dates and genotypes. The phases recorded have been: E=emergence, F1= main inflorescence appearance A1= flowering main raceme, G1/1= beginning of main raceme fruit set, G1/2= end of main raceme fruit set, M1= main raceme maturation.

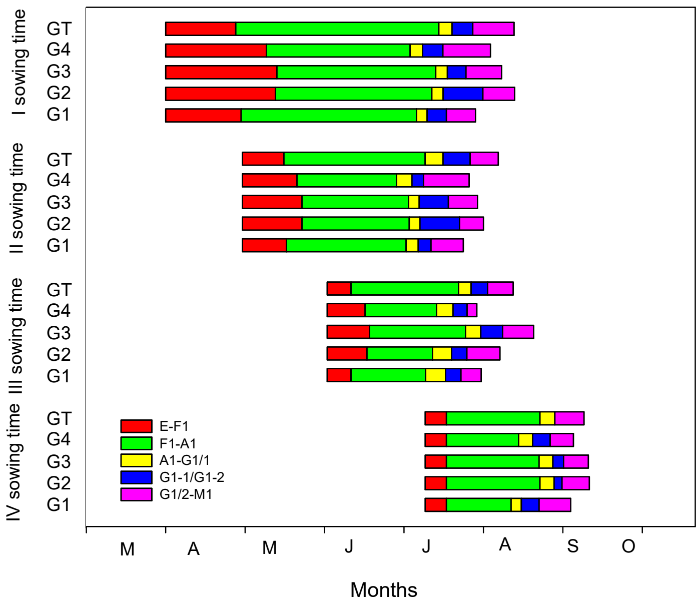

Figure 2. Length of period between phenological phases for the production of the primary raceme (E=emergence; F1 = main inflorescence appearance; A1 = Flowering main raceme; G1 / 1 = Beginning of main raceme fruit set; G1 / 2 = End of main raceme fruit.

3.3 Results and discussions

The lengths of the phenological phases decrease by shifting the sowing dates. The first sowing S1, sown in April, has a growing season that goes from April to late August /beginning of September with a duration of the season of about 5 months; while the last sowing S4, sowed in mid of July has a growing season of about 3 months. Furthermore, the flowering of the first three sowing dates were all recorded late July due to the high temperatures and photoperiodic effect.

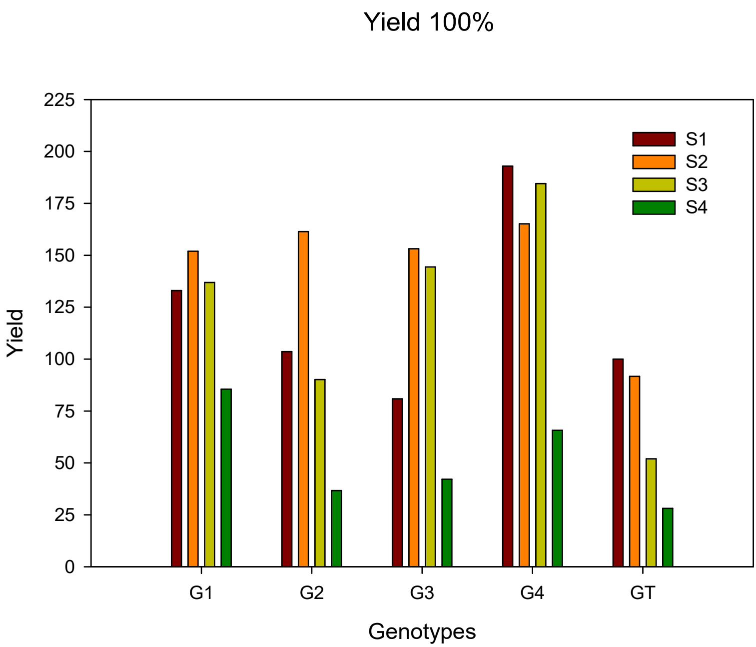

Figure 3. Relative seed yield in relation to sowing dates and genotypes referred to total yield of GT S1(100= 1601.13 kg/ha^{-1}).

By referring our local variety GT S1 as 100 = 1601, 131 kg ha^{-1}, on average the total highest yield were shown for S2 and S3 for primary and secondary racemes (Figure 3). The annual Kaiima dwarf hybrids genotypes produced almost the double in comparison with our poliannnual local variety. Among them, the genotype which has produced the most is the G4 with a yield of 52% more than the others.

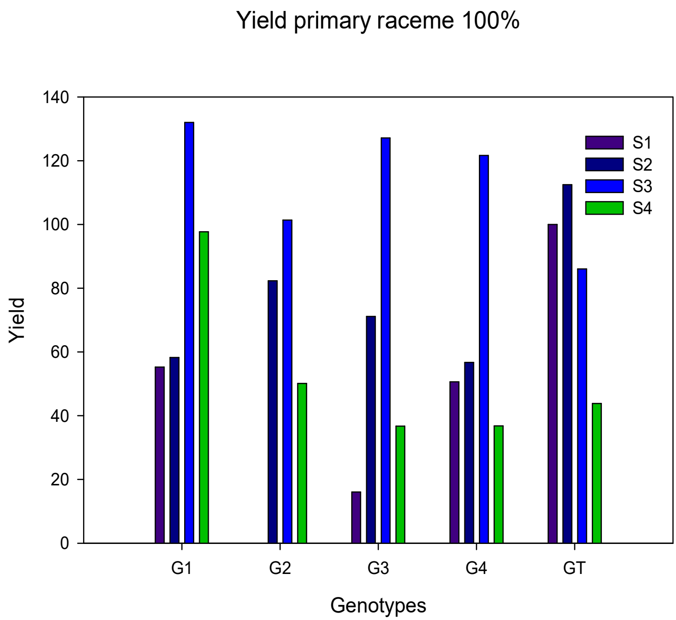

Figure 4. Relative seed yield of primary raceme in relation to sowing dates and genotypes referred to total yield of GT S1 (100= 967.91 kg ha^{-1})

According to several studies the contribution of primary racemes on the total amount of yield varies from 14 to 69%

(Chakrabarty et al., 2021). Even for this experiment the yield from the main raceme is lower than the yield of the secondary racemes. On average the genotype that has higher yield is our local variety GT who produced the most on the main racemes with the highest percentage of 112% in S2. (Figure. 4).

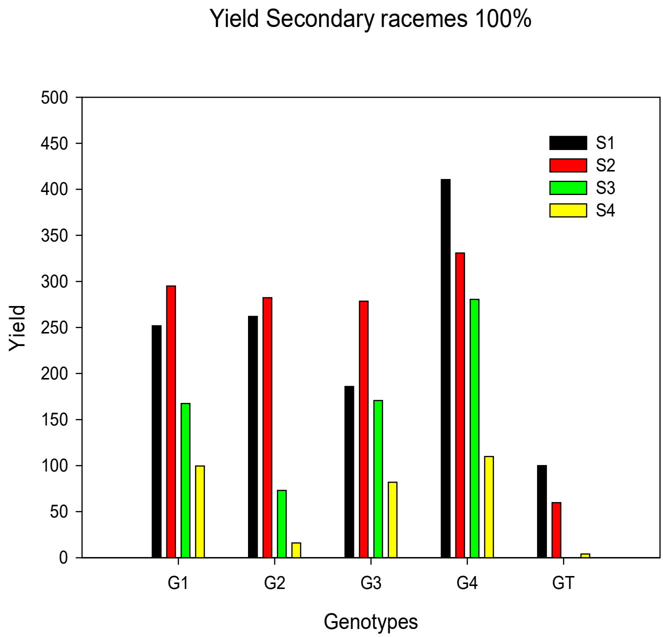

Figura 5. Relative seed yield of secondary raceme in relation to sowing dates and genotypes referred to total yield of GT S1 (100= 633.2 kg/ha^{-1})

The average GT yield of the secondary racemes is lower than the GT yield of primary racemes (Figure 5). This can be explained by the shifting of the sowing dates that reduce the growing season for our local variety. While Kaiima hybrids genotypes have an average yield of the secondary racemes higher than in the primary racemes. By referring our local variety GT S1 as 100 = 633, 2 kg ha^{-1}, the hybrid with a higher percentage is confirmed to be G4 with an average of 282% of yield.

3.4 Conclusions

The field experiment conducted on irrigated castor bean carried out in the south of Italy have shown that the lengths of the growing season has decreased by shifting the sowing dates.

The flowering of the first three sowing dates (S1, S2, S3) was recorded around the end of July thanks to the high temperatures and the photoperiodic effect.

Interesting yields were registered for the yield of the hybrids which showed almost double yield compared to the local variety. In particular, the G4 hybrid proved to be the most interesting hybrid to adapt to the Mediterranean environment.

While the local variety showed higher yields for primary raceme production, hybrids registered higher yields for secondary racemes, this can be explained by the shifting of the sowing dates which reduces the growing season, not allowing our variety to develop properly

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4 Evaluation Effects of Nitrogen Fertilization and Soil Water Content on Seed and Oil Yield in Perennial Castor in a Mediterranean Environment

Based on: Calcagno S., Piccitto A., Patanè C., Cosentino S.L., Testa G. Effects of Nitrogen Fertilization and Soil Water Content on Seed and Oil Yield in Perennial Castor in a Mediterranean Environment. Under review on *Agronomy.*

Effects of Nitrogen Fertilization and Soil Water Content on Seed and Oil Yield in Perennial Castor in a Mediterranean Environment

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ABSTRACT: Castor (*Ricinus communis* L.) is an oilseed species that can be grown as a semi-perennial in the Mediterranean environment including the coastal areas of Sicily.

The present study investigates the optimization of the cultivation technique of castor, keeping the crop over a two-year period, through the evaluation of different agronomical inputs in order to increase seed yield. The effects of irrigation-I and nitrogen fertilization-N on seed and oil yield and their components, were assessed in castor cultivated in a typical semi–arid environment. Four levels of irrigation (I0, I30, I60, I100: 0, 30, 60, 100% of crop evapotranspiration-ETm restoration, respectively), as main-plot, and three levels of nitrogen fertilization (N) (0, 60, 120 kg N ha^{-1}), as sub-plot, were considered.

Irrigation mostly affected number of racemes per plant, number of capsules per raceme, and seed weight.

Oil content was on average 39.2 % and 45.6 % for the first and second year, respectively. The highest seed yield was obtained by I100N120 treatment (4154.0 kg ha^{-1}), however the combination of a high soil nitrogen level (N120) and medium water availability (I60) resulted in satisfactory seed and oil yield.

Keywords: *Ricinus communis* L.; seeds yield; oil yield; Mediterranean environment;

4.1 Introduction

Castor (*Ricinus communis* L.) is a non-food, drought-resistant, energy crop that has been used since antiquity because of the distinctive properties of its oil, with evidence of its use dating back to ancient Egypt (Scarpa and Guerci, 1982; Franz, 1988).

Castor is a member of the Euphorbiaceae family that is found across all the tropical and semi-tropical regions of the world (Weiss, 2000). Ethiopia is considered to be the most probable site of origin because of the presence of high diversity (Moshkin, 1986; Anjani, 2012; Fernández-Martínez et al., 2012).

Castor crop due to its ease of cultivation, drought and salinity tolerance, and adaptability to a variety of growing conditions, is one of the most important non-food crops grown in arid and semiarid areas (Babita et al., 2010; Nahar, 2013).

The oil content of castor seed ranges between 40% and 60% and the oil is composed of the following fatty acids: ricinoleic acid (91-95%), linoleic acid (4-5%), and palmitic and stearic acids (1-2%) (Severino and Auld, 2013a). Castor oil has a growing international market, as evidenced by over 700 applications ranging from medicines and cosmetics to the replacement of petroleum in the production of plastics, lubricants, and biodiesel (Ogunniyi, 2006; Dias et al., 2013; Keera et al., 2018).

Several studies evidenced the adaptability of castor to European soil and climate (Arnaud, 1990; Baldanzi and Benvenuti, 1995; Laureti and Marras, 1995; Koutroubas et al., 1999); moreover, castor was extensively grown in Europe until the 80s', and wild types spontaneously grow in many areas of the Mediterranean region.

In semiarid environments of southern Italy, Castor behaves as an annual spring-summer crop that requires irrigation, whose sowings are typically performed in April, when the soil temperature reaches a stable level of 16-17 °C, ensuring rapid seed germination and a uniform crop establishment (Weiss, 1983; Falasca et al., 2012, Zanetti et al. 2017).

However, Mediterranean climate of lowlands and coastal areas of Sicily, presents cool winter with infrequent light frost and peak of annual precipitation during autumn and winter. In these areas, castor can be grown as semiperennial, extending the growth season during a period of the year when rainfalls are adequate to the crop, thus reducing the irrigation requirements (Patanè et al., 2019).

Moreover, winter mild temperatures extend the length of the growing season, allowing a greater vegetative development increasing the potential yield (Falasca et al., 2012; Ramanjaneyulu et al., 2013).

Preliminary field experiments carried out in southern Sicily and in Tunisia have shown the possibility of exploiting the perennial habit of this species (Anastasi et al., 2015).

The present study was conducted in a local type of castor selected from a Tunisian genotype by the Department of Agriculture, Food and Environment (Di3A) of University of Catania (Italy). The aim of the study was the optimization of the cultivation technique in castor, through the assessment of the crop response in terms of seed and oil yield, to different levels of irrigation and nitrogen supply, keeping the crop over a two - year period.

4.2 Materials and Methods

4.2.1 Field experiment

The field experiment was conducted for two successive years (2021 and 2022) at the Experimental farm of the University of Catania, Italy (10 m a.s.l., 37°25’ N lat., 15° 03’ E long.) in a typical xerofluvent soil. The soil characteristics were: clay 32.3%, sand 55.9%, silt 11.8%, organic matter 1.4%, pH 7.6, total N 0.2%, available P_2O_5 46.1 mg kg^{-1}, ex-changeable K_2O 293.3 mg kg^{-1}.

The soil of the experimental area was ploughed before sowing and fertilized with 70 kg ha^{-1} P_2O_5 (as single superphosphate). The experiment was set up with a split-plot experimental design, with a 4 × 3 factorial schemes consisting of four irrigation levels (I0, I30, I60, I100, respectively 0, 30, 60, 100% of crop maximum evapotranspiration - ETm restoration), three mineral nitrogen (N) fertilization rates (N0, N60, N120, respectively 0, 60, 120 kg N ha^{-1}), and three replicates per treatment. Irrigation was assigned to the main plot and N fertilizer to the sub-plot. The amount of nitrogen for each N-level was applied in the first year as followed: 35% before sowing (as ammonium sulphate), 45% before flowering (as ammonium nitrate), and 20% at the beginning of grain formation (as ammonium nitrate). Each plot measured 68 m^2 (8 x 8.5 m) with 1.7 m row to row spacing and 1 m plant to plant distance (sowing density 0.58 plants per $1m^2$) and consisted of five rows.

Seeds of a local Tunisian genotype of castor selected by the Department of Agriculture, Food and Environment (Di3A) of University of Catania (Italy) Di3A were sown manually on the 15^{th} of April 2021 by placing three seeds per hole at 3 to 4 cm depth.

Plots were hand thinned to one plant per hole when plants were at the four leaf stage.

A drip irrigation system using 16 mm pipes was installed to irrigate the plants, supplying 2436, 1604, 873 m^3 ha^{-1} of water, in 2021, and 1624, 1070, and 582 m^3 ha^{-1} of water, in 2022, to I100, I60, I30, respectively. Plots in I0 were irrigated up to seedling establishment with total 237 m^3 ha^{-1} of water.

The irrigation volume (V) was determined as the maximum available soil water content in a 0.6 m soil depth where root system is predominantly developed.

Irrigation was scheduled when the sum of daily maximum crop evapotranspiration (ETm) corresponded to V, subtracting rainfall events from the calculation.

Daily ETm was calculated according to the following formula:

$$ETm = E_0 \times Kp \times Kc$$

where ETm is the maximum daily evapotranspiration (mm); E_0 is the evaporation of class-A pan (mm); Kp is the pan coefficient, equal to 0.80 in semi-arid environment. The following crop coefficients (Kc) were considered in the calculation: 0.4 from emergence to the four leaf stage; 0.7 from the four-leaf stage to the beginning of flowering; 1.2 from the beginning of flowering to complete capsule development of the first raceme.

The irrigation volume was calculated according to:

$$V = 0.66 \times (FC - WP) \times \phi \times D \times 10^3$$

where V = water amount (mm); 0.66 = readily available water not limiting for evapotranspiration; FC = soil water content at field capacity (27% of dry soil weight); WP = soil water content at wilting point (11% of dry soil weight); ϕ = bulk density (1.1 g cm^{-3}); and D = rooting depth (0.6 m).

For each irrigation level, the crop water use (CWU) was determined by means of a water balance from plant emergence to the start of rainy season (first year = *i*) and from plant re-growth up to the last irrigation (second year = *ii*)

$$CWU_{(i)(ii)} = I_{(i)(ii)} + P_{(i)(ii)} \pm \Delta C_{(i)(ii)} \qquad (3)$$

where $CWU_{(i)(ii)}$ = crop water use (mm); $I_{(i)(ii)}$ = water supplied by means of irrigation (mm); $P_{(i)(ii)}$ = precipitation (mm); $\Delta C_{(i)}$ =

difference between soil water content at plant emergence and soil water content after the last irrigation (mm); $\Delta C_{(ii)}$ = difference between soil water content at plant re-growth and soil water content after the last irrigation (mm).

Soil samples were collected to a depth of 0.6 m in each treatment, before the sowing and at anthesis stage during the first year. During the second growing season, soil samples were collected before plant re-growth and at anthesis stage. Fresh soil samples were immediately weighted, closed in plastic zip bags and then transferred in a ventilated oven to dry at 105 °C up to a constant weight.

Daily meteorological data and potential evapotranspiration (ET_0) were continuously measured through a weather station connected to a data logger (Delta-T, WS-GP1 Compact) and a Class A evaporation pan (mm d^{-1}).

In the first year (2021), during the growing season, the date of occurrence of the main phenological stages of crop (Rios et al., 2016): seedling emergence (Ve), anthesis (A) and physiological maturation (M) was recorded, and physiological plant gas exchange (photosynthesis and transpiration), using a portable system (LCi-SD, ADC BioScientific, Great Amwell, Hertfordshire, UK) were measured, at 7 -10 day intervals for total 9 measurements.

Harvest was carried out starting from the primary racemes as follows: 7 September 2021, 25 September 2021, 20 October 2021 and 20 November 2021 for I100, I60, I30, I0, respectively.

The secondary racemes were harvested on 02 October, 22 October, 18 November and 10 December of the same year, respectively in I100, I60, I30, I0, according to the different flowering period.

In the second year (2022), the harvests were performed starting from 10 June until 25 July, according to the different flowering period.

At harvest, 18 plants (six plants from the three central rows) of each plot were collected.

In the laboratory, the first and the secondary racemes were kept separated and weighed; the capsules were counted and manually separated from peduncle, and seeds were separated from capsules to measure the number of capsules per raceme and the seed weight per raceme.

Water use efficiency (WUE) was calculated as the ratio between the seed yield (kg ha^{-1}) and crop water use (CWU), considering the total of yields and the total of water used, in the two years.

4.2.2 Meteorological course

Weather conditions during the field experiment were those typical of a Mediterranean climate.

A total of 94 mm and 50.4 mm of rainfall were recorded respectively before sowing (January to April 2021) and from sowing to the 1^{st} harvest (April to September 2021).

Cumulative rainfall from September to December 2021 was 630.20 mm. In the same year, total ET_0 was 1018.83 mm, with an average of 3.8 mm day^{-1}, and average temperatures were 26.2°C (T max), 15.5°C (T min), and 20.7 °C (T mean) (Figure 1).

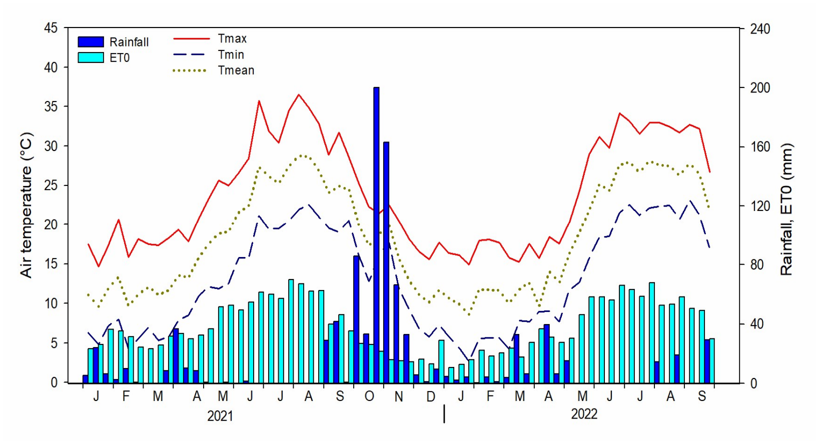

Figure 1. Meteorological course (air temperature, rainfall and reference evapotranspiration-ET0) recorded during the 2-year period (2021–2022) at the Experimental farm of the University of Catania, Italy (10 m a.s.l., 37°25’ N lat., 15°03’ E long.).

During the second growing season (January - July 2022) rainfall was 114.6 mm and ET_0 was 679.9 mm, with an average of 3.5 mm day^{-1}. In the same year, annual average temperatures were 21.8°C (T max), 10.7°C (T min), and 16.3°C (T mean).

4.2.3 Oil extraction

The seeds were grinded to be subjected to the oil extraction using the GM200 blade mill (Retsch). The oil content was determined according to Randall method, by the use of a quantitative solvent extractor SER 148/6 (Velp Scientifica) (Randall, 1974).

The extraction was performed by immersion of the cellulose thimble containing 3 g of sample in the boiling n-Hexane solvent, followed by a rinsing phase and a recovery phase. Extraction vessels were placed in an oven at 100 °C for 30 minutes, to eliminate any solvent residues. The analyses were performed in triplicate.

4.2.4 Statistical Analysis

Data on phenological stages, morphological trats, seed and oil yield and components, were subjected to statistical analysis using ANOVA according to the experimental design.

Before conducting the ANOVA, the Bartlett's test was run to verify the assumption of homogeneity of variances. A LSD's post hoc test was used for mean separation at $P \leq 0.05$.

Data were statistically analyzed using CoHort Software (CoStat version 6.003, Monterey, CA, USA).

4.3 Results

4.3.1 Length of phenological stages

Seedling emergence occurred 15-17 days after sowing. The ANOVA showed a significant effect of irrigation and N-fertilization on the time-intervals emergence-anthesis (E-A) and anthesis-seed physiological maturation of primary raceme (A-M1). Across N levels, plants in I100 flowered first (about 68 days after sowing), while those in I0 flowered last (117 days after sowing). Anthesis started 91 and 79 days after sowing, respectively in I30 and I60. The physiological maturation of the primary raceme was completed 145 days after sowing, in I100, and 219 days after sowing, in I0. In I30 and I60, plants ripened respec-tively in 188 and 163 days.

The length of the interval between the physiological maturation of primary raceme and that of secondary racemes (M1-M2) significantly changed with the level of irrigation.

Secondary racemes completed maturation 25, 34, 36 and 38 days after primary raceme, in I100, I60, I30, and I0, respectively (Figure 2). Low levels of soil water availability led to a delayed anthesis and seed ripening as well as the increasing of nitrogen supply.

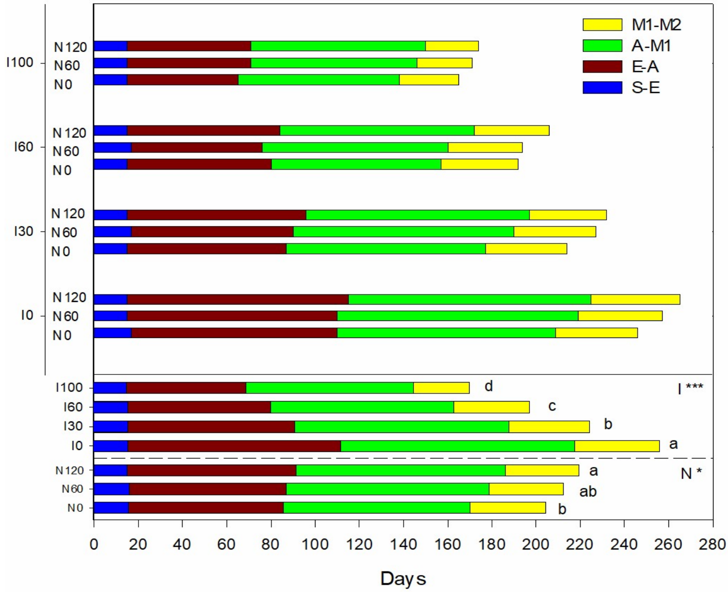

Figure 2. Length of phenological stages in castor (sowing-emergence (S-E), emergence-anthesis (E-A), anthesis-physiological maturation of primary raceme (A-M1), maturation of primary raceme-maturation of secondary racemes (M1-M2)) in relation to irrigation (I0, I30, I60, I100% = 0, 30, 60, 100% ETm restoration, respectively) and N-fertilization (N0, N60, N120 = 0, 60, 120 kg ha^{-1} of N, respectively). The lower part shows mean separation of total phenological stages length, with different letters representing statistically significant means according to the LSD test ($p \leq 0.05$). Significance levels of ANOVA for irrigation (*** significant at $p \leq 0.001$) and N- fertilization (* significant at $p \leq 0.05$) are shown.

4.3.2 Morphological traits, yield and yield components

Most of the productive traits, such as number of capsules per raceme, seed yield and seed weight, were significantly affected by the studied factors. The effects of the different irrigation and nitrogen levels on these traits are reported in Tables 1 - 5.

The number of secondary racemes per plant, which was greater in the second year (2022) (up to 11.8 in I100N120), was significantly affected by both factors, but to greater extent by the irrigation ($p \leq 0.001$) than N ($p \leq 0.05$) level. In both years, more secondary racemes were measured in plants under full irrigation (I100) and in those fertilized with the highest rate of N (N120). In 2021, increasing N availability even had an adverse effect under no irrigation, significantly decreasing the number of racemes at the highest N rate (Table 1). In the second year, increasing N rate was beneficial on this trait only in I0 and, to a lesser extent, in I100 (Table 1).

In 2021, the number of capsules on primary raceme was significantly affected by the level of water supply and N applied. Increasing N had a greater effect under I0 and I100 (I x N, $p \leq 0.001$), as a result, plants in I0N0 and I100N120 had respectively the lowest (21.0) and the highest (66.2) number of capsules per raceme (Table 2).

Table 1: Number of secondary racemes in relation to irrigation (I0, I30, I60, I00 = 0, 30, 60, 100% ETm, respectively) and N level (N0, N60, N120 = 0, 60, 120 kg ha^{-1} N) (year 2021-2022). Average values of all N levels (last column) and average values of all irrigation levels (last row) followed by the same letter do not significantly differ at $p \leq 0.05$.

Number of secondary racemes (2021)						
	I0	I30	I60	I100	Mean	Significance
N0	2.0	2.0	1.4	3.5	2.2 ab	I ***
N60	2.5	1.5	2.0	2.5	2.1 b	N *
N120	1.0	2.0	2.0	4.0	2.3 a	I x N ***
Mean	1.8 b	1.8 b	1.8 b	3.3 a		$LSD_{I \times N} = 0.4$
Number of secondary racemes (2022)						
	I0	I30	I60	I100	Mean	Significance
N0	4.2	10.7	8.8	9.3	8.2 b	I ***
N60	5.5	8.5	7.8	10.3	8.0 b	N *
N120	8.4	6.0	9.8	11.8	9.0 a	I x N ***
Mean	6.1 c	8.4 b	8.8 b	10.5 a		$LSD_{I \times N} = 1.3$

*, *** Significant at $p \leq 0.05$ and 0.001, respectively.

The number of capsules on secondary raceme in 2021 was significantly affected by the level of N and I applied. Significant interaction ($p \leq 0.001$) revealed a changing effect of N depending on the irrigation regime.

As a result, the I60N0 and I60N120 treatments reported, respectively, the lowest (25.5) and the highest (47) number of capsules per raceme. In 2022, the number of capsules on secondary racemes, quite lower than that measured in 2021, was also affected by both factors (I, N, $p \leq 0.001$). Nitrogen and irrigation significantly interacted on this trait in 2022, as well, and the effect of increasing N level was evident in I60 and I100, but not in I0 and I30.

In this year, maximum (30) and minimum (10) number of capsules were measured respectively in I100N120 and I0N0 (Table 2).

Table 2: Number of capsules in relation to irrigation (I0, I30, I60, I00 = 0, 30, 60, 100% ETm, respectively) and N level (N0, N60, N120 = 0, 60, 120 kg ha^{-1} N) (year 2021-2022). Average values of all N levels (last column) and average values of all irrigation levels (last row) followed by the same letter do not significantly differ at $p \leq 0.05$.

Number of capsules						
Primary raceme (2021)						
	I0	I30	I60	I100	Mean	Significance
N0	21.0	24.2	26.0	59.0	32.5 c	I ***
N60	45.0	34.0	30.0	43.3	38.1 b	N ***
N120	36.0	31.0	29.5	66.2	40.7 a	I x N ***
Mean	34.0 b	29.7 c	28.5 c	56.2 a		LSD $_{I x N}$ = 6.0
Secondary racemes (2021)						
	I0	I30	I60	I100	Mean	Significance
N0	29.0	32.5	25.5	32.0	29.8 b	I ***
N60	32.0	43.5	36.0	41.0	38.1 a	N ***
N120	36.0	35.5	47.0	38.0	39.1 a	I x N ***
Mean	32.3 b	37.2 a	36.2 a	37.0 a		LSD $_{I x N}$ = 5.4
Secondary racemes (2022)						
	I0	I30	I60	I100	Mean	Significance
N0	10.0	17.0	20.0	22.0	17.3 b	I ***
N60	27.0	18.0	21.0	27.0	23.3 a	N ***
N120	22.0	16.0	23.0	30.0	22.8 a	I x N ***
Mean	19.7 c	17.0 d	21.3 b	26.3 a		LSD $_{I x N}$ = 3.4

*** Significant at p ≤ 0.001

The weight of seed measured on primary raceme in 2021 was positively affected by the increasing level of N, while the effect of irrigation on this trait was less clear. The weight ranged between 0.23 g (I100N0) and 0.48 g (I0N120). A significant interaction between the two experimental factors was found at ANOVA (Table

3).

The seed weight in secondary racemes was slightly higher than that measured in primary raceme, and ranged between 0.27 g (I60N120) and 0.58 g (I100N60), in 2021, and 0.27 g (I0N0) and 0.43 g (I60N0 and I100N120), in 2022 (Table 3).

Table 3: Seed weight (g) in relation to irrigation (I0, I30, I60, I00 = 0, 30, 60, 100% ETm, respectively) and N level (N0, N60, N120 = 0, 60, 120 kg ha^{-1} N) (year 2021-2022). Average values of all N levels (last column) and average values of all irrigation levels (last row) followed by the same letter do not significantly differ at $p \leq 0.05$.

Seed weight (g)						
Primary raceme (2021)						
	I0	I30	I60	I100	Mean	Significance
N0	0.32	0.24	0.46	0.23	0.31 c	I ***
N60	0.34	0.42	0.44	0.31	0.38 b	N ***
N120	0.48	0.44	0.40	0.35	0.42 a	I x N ****
Mean	0.38 b	0.37 b	0.44 a	0.30 c		$LSD_{I \times N} = 0.1$
Secondary racemes (2021)						
	I0	I30	I60	I100	Mean	Significance
N0	0.36	0.38	0.49	0.39	0.41 b	I ***
N60	0.40	0.37	0.55	0.58	0.47 a	N ***
N120	0.41	0.40	0.27	0.42	0.37 c	I x N ****
Mean	0.39 b	0.38 b	0.44 a	0.46 a		$LSD_{I \times N} = 0.1$
Secondary racemes (2022)						
	I0	I30	I60	I100	Mean	Significance
N0	0.27	0.29	0.43	0.32	0.33 b	I ***
N60	0.28	0.35	0.39	0.33	0.34 b	N ***
N120	0.38	0.36	0.38	0.43	0.39 a	I x N ****
Mean	0.31 b	0.33 b	0.40 a	0.36 b		$LSD_{I \times N} = 0.1$

*** Significant at $p \leq 0.001$

Both experimental factors significantly affected seed yield ($p \leq 0.001$), as total of the two years and of all racemes (primary and secondary), which progressively increased with the raise of soil water availability and N fertilization rate. As a result, seed yield was minimum in I0N0 (641.1 kg ha^{-1}) and maximum in I100N120 (4154.0 kg ha^{-1}) (Figure 3). The two experimental factors significantly interacted on crop productivity ($p \leq 0.001$). Indeed, while under no irrigation, the beneficial effect of N-fertilizer on seed yield was the same irrespective of the N-level (60 or 120 kg ha^{-1}), thus no further yield increase occurred in N120 respect to N60, in all irrigated treatments (from I30 to I100), the promoting effect of N was progressive from 0 to 120 kg ha^{-1}.

Seed yield of primary raceme picked in I100N120 (506.6 kg ha^{-1}). The lowest yield corresponded to I30N0 (95.6 kg ha^{-1}). Seed yield of secondary racemes was 313.1 kg ha^{-1} in I0N0 and 1623.8 kg ha^{-1} in I100N120. In the second season, 2023.6 and 230.5 kg ha^{-1} were produced respectively under the most (I100N120) and the least (I0N0) favorable soil conditions.

Relative contribution of primary and secondary racemes slightly changed within the different I x N combinations. Overall, secondary racemes produced in the second year contributed to greater extent to total seed yield than those of primary and secondary racemes produced in the first year.

The yield increase in plants in plots irrigated over those cultivated under rainfed conditions was the result of an increased number of secondary racemes (R^2= 0.948***, data not shown).

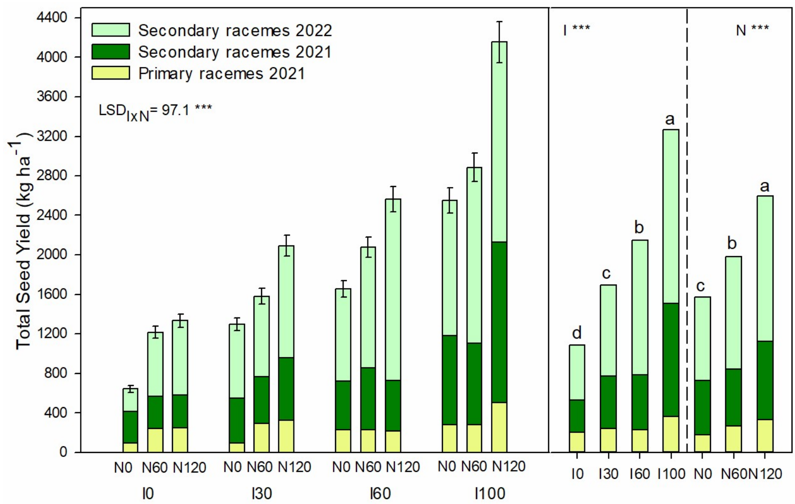

Figure 3. On the left, total seed yield (kg ha^{-1}) (primary and secondary racemes of 2021 and 2022) in relation to irrigation (I0, I30, I60, I00 = 0, 30, 60, 100% ETm, respectively) and N-fertilization (N0, N60, N120 = 0, 60, 120 kg ha^{-1} N) with error bars indicating the standard error. On the right, mean separation of total seed yield, with different letters representing statistically significant means according to the LSD test ($p \leq 0.05$). Significance levels of ANOVA for irrigation and N- fertilization (*** significant at $p \leq 0.001$) and significant interaction (LSD $_{IxN}$ at $p \leq 0.05$) are shown.

4.3.3 Oil content and oil yield

Seed oil content was affected by both irrigation and N levels.

In the first year, oil content in the primary raceme was minimized in I100 (36.1%, on average of N levels) and maximized in I60 (45.5%) (I, $p \leq 0.001$) (Table 4). Across the irrigation treatments, oil content was the greatest (42.8%) and the lowest (36.7%) in N60 and N0, respectively. Similarly, oil content in secondary racemes, which was not much different than measured in primary raceme, was the highest in N60 (40.5%) but picked in I100 (44.4%).

The two experimental factors significantly interacted on this trait ($p \leq 0.001$, for both primary and secondary racemes). In primary raceme, maximum content was registered in I60N60 (47.2%), and that minimum in I0N0 (30.4%). In secondary racemes, oil content ranged between 29.6% (I0N0) and 46.3% (I100N60). In the second year, oil content, which was slightly higher than that measured in 2021, ranged from 42.4% (I100N120) to 50.6% (I0N0) (Table 4).

No effect of N fertilization was highlighted at ANOVA. Differently, oil content was adversely affected by irrigation (I, $p \leq 0.01$), significantly decreasing in I30, I60, and I100 (44.6 on average) respect to I0 (48.5%). The two factors did not interact on this trait in 2022.

I x N treatments ranked for oil yield according to seed yield. As a result, plants of I100 were the most productive in terms of oil yield, despite lower seed oil content, with maximum oil yield achieved in I100N120 in 2021 (853.3 kg ha^{-1}) and 2022 (858.2 kg ha^{-1}) (Figure 4). I0 and I30 treatments produced the lowest oil yields, despite a high seed oil content.

Table 4: Oil content (%) in seeds of primary and secondary racemes in relation to irrigation (I0, I30, I60, I00 = 0, 30, 60, 100% ETm, respectively) and N level (N0, N60, N120 = 0, 60, 120 kg ha^{-1} N) (year 2021-2022). Average values of all N levels (last column) and average values of all irrigation levels (last row) followed by the same letter do not significantly differ at $p \leq 0.05$.

Oil content (%)						
Primary raceme (2021)						
	I0	I30	I60	I100	Mean	Significance
N0	30.4	35.2	45.3	35.8	36.7 c	I ***
N60	44.0	42.1	47.2	37.8	42.8 a	N ***
N120	40.6	44.0	44.0	34.5	40.8 b	I x N ***
Mean	38.4 c	40.4 b	45.5 a	36.1 d		$LSD_{I \times N} = 5.9$
Secondary racemes (2021)						
	I0	I30	I60	I100	Mean	Significance
N0	29.6	37.2	36.5	45.3	37.2 b	I ***
N60	38.9	37.1	39.8	46.3	40.5 a	N ***
N120	40.3	38.3	30.8	41.8	37.8 b	I x N ***
Mean	36.3 b	37.6 b	35.7 b	44.4 a		$LSD_{I \times N} = 6.0$
Secondary racemes (2022)						
	I0	I30	I60	I100	Mean	Significance
N0	50.6	46.2	44.2	43.6	46.2 a	I **
N60	46.5	45.8	46.5	46.1	46.2 a	N ns
N120	48.4	43.6	43.1	42.4	44.4 a	I x N ns
Mean	48.5 a	45.2 b	44.6 b	44.0 b		

, * Significant at $p \leq 0.01$ and $p \leq 0.001$, rescpectively; ns not significant

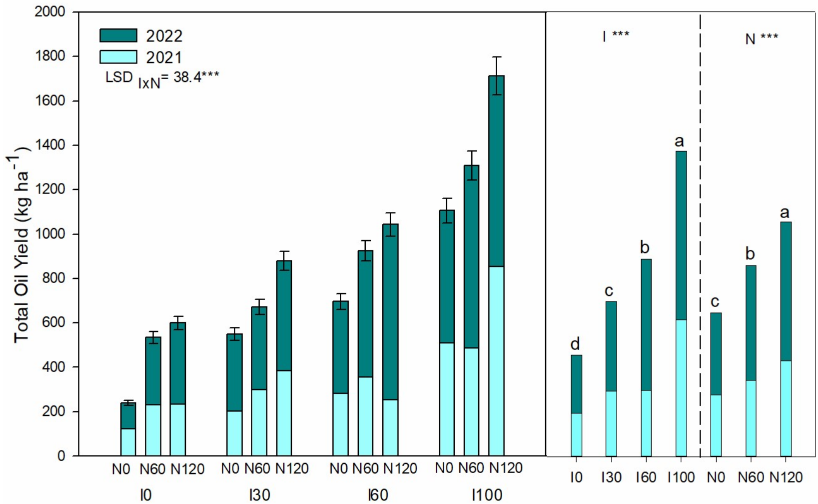

Figure 4. On the left, total oil yield (kg ha^{-1}) of two years (2021 and 2022) in relation to irrigation (I0, I30, I60, I00 = 0, 30, 60, 100% ETm, respectively) and N-fertilization (N0, N60, N120 = 0, 60, 120 kg ha^{-1} N) with error bars indicating the standard error. On the right, mean separation of total oil yield, with different letters representing statistically significant means according to the LSD test ($p \leq 0.05$). Significance levels of ANOVA for irrigation and N- fertilization (*** significant at $p \leq 0.001$) and significant interaction (LSD_{IxN} at $p \leq 0.05$) are shown.

4.3.4 Water use efficiency

Water use efficiency was calculated by dividing the seed yield (kg ha^{-1}), as total of primary and secondary racemes obtained in the two years, and crop water use (mm), considering the total of water used in the two years.

The crop used the water more efficiently at high N levels and at low irrigation levels. Indeed, WUE significantly and progressively improved with the raise in N-fertilization rate, from 0.55 (N0) to 0.96 kg ha^{-1} mm (N120) (Table 5). As expected, WUE was maximized under no irrigation (1.03 kg ha^{-1} mm), progressively declining with irrigation, down to 0.61 kg ha^{-1} mm (I60). No further decrease in WUE occurred when irrigation rate was increased from 60 to 100% ETm (0.66 kg ha^{-1} mm).

The significant interaction *I x N* is related to the lack of significant difference between I60 and I100 in the N60 treatment. However, the least variability between the highest and the lowest value was observed in N0 treatment (22%) as compared to N60 and N120 treatments (49% and 43%, respectively).

Table 5: Water use efficiency in relation to irrigation (I0, I30, I60, I00 = 0, 30, 60, 100% ETm, respectively) and N level (N0, N60, N120 = 0, 60, 120 kg ha^{-1} N) (year 2022). Average values of all N levels (last column) and average values of all irrigation levels (last row) followed by the same letter do not significantly differ at $p \leq 0.05$.

Water use efficiency- WUE (kg ha^{-1} mm^{-1})						
	I0	I30	I60	I100	Mean	Significance
N0	0.62	0.58	0.48	0.52	0.55 c	I ***
N60	1.18	0.70	0.60	0.59	0.77 b	N ***
N120	1.29	0.93	0.74	0.86	0.96 a	I x N ***
Mean	1.03 a	0.74 b	0.61 d	0.66 c		$LSD_{I \times N} = 0.04$

4.3.5 Photosynthesis and transpiration rate

Photosynthesis and transpiration rates were measured from June to August in the first year (2021). N-fertilisation seem do not affect photosynthesis. Indeed, it did not change with the N level, keeping values quite constant along the growing season (Figure 5C).

Differently, in relation to the irrigation, a tendency of photosynthesis to increase with time was observed in plants well watered (I100) (Figure 5A). Interestingly, photosynthesis under moderate deficit irrigation (I60) did not much fluctuate, whilst under moderate stress conditions (I30) it was kept constant but tended to decrease in the last measurement (that August). Under no irrigation (I0), photosynthesis progressively declined from late June onwards.

A similar response to N-fertilization and irrigation levels was observed in leaf transpiration. This last picked in late July, when plants of latest treatments (those not irrigated) were approximately at the maximum growth period (Figure 5B, D). Afterwards, leaf transpiration steeply declined to the initial level, and was maintained more or less constant until late August. Overall, plants in I0 transpired less than those irrigated.

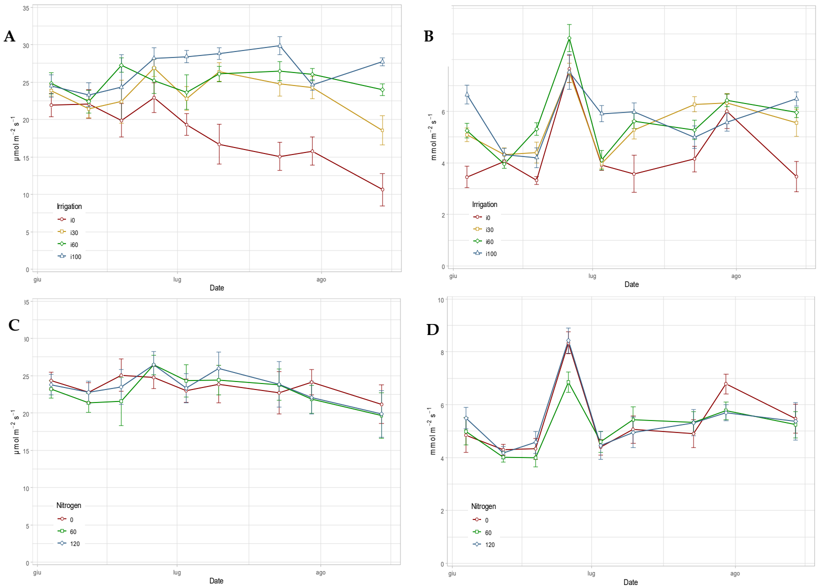

Figure 5. A) Net photosynthesis (µmol CO_2 m^{-2} s^{-1}) according to the irrigation level and C) nitrogen fertilization levels. B) Transpiration rate (mmol H_2O m^{-2} s^{-1}) according to the irrigation level and D) nitrogen fertilization levels.

4.4 Discussion

The irrigation was found to reduce the length of plant life cycle, influencing the length of phenological stages. Low levels of soil water availability induced a delay in anthesis and consequently seed ripening as well as the increasing of nitrogen supply.

The physiological maturation of the primary raceme was completed 219 days after sowing in plants of unirrigated plots (those of I0), while plant fully watered (those of I100) completed the maturity in 145 days, only. These results matched those of Patanè et al. (2019) who reported 240 days (with sowing in November) to 99 days (with sowing in May) occurring from sowing to maturity, in castor cultivated in coastal areas of Sicily.

Number of racemes per plant, number of capsules per raceme, and seed weight, are the main traits investigated in castor breeding programs, since several studies reported a positive correlation of these traits to final seed yield (Ramesh and Venkate, 2001; Lakshmamma et al., 2005).

The yield components were influenced by irrigation in a different way. In the first year, the I0 and I30 had few racemes per plant, low number of capsules per raceme and low seed weight. From I60 to I100, the number of racemes per plant increased from 1.8 to 3.3, and the number of capsules per primary raceme increased from 28.5 to 56.2. Data of the second year confirmed a low number of secondary racemes and capsules per raceme, and low seed weight, in I0 and I30. From I60 to I100 treatment, the number of secondary racemes increased from 8.75 to 10.5 and the number of capsules per raceme increased from 21.3 to 26.3.

Irrigation is the main factor influencing seed yield. In fact, the data showed that maximum number of capsules per plant (66.2 and 30.0 in the first and second year) was obtained in I100N120.

Moreover, higher soil water availability induced higher photosynthesis rate and consequently higher seed production.

Nitrogen fertilization had a positive effect on the seed yield in primary raceme, but induced a delay in the development of secondary racemes, causing a reduction of mean seed yield for secondary racemes. Average seed yield (1270.5 kg ha^{-1}) was within the range reported by Anastasi et al. (2015) for Sicilian genotypes of castor.

The contribution of primary and secondary racemes on total yield was not the same, in relation to nitrogen and water availability.

On average, the contribution to the yield of primary racemes was 30% and that of the secondary ones 70%, according to Severino et al. (2013). Irrigation and N-fertilization influenced the number of secondary racemes per plant. High levels of water availability promoted the development of secondary racemes. High soil nitrogen level combined with low water availability extended the vegetative growth delaying the formation of secondary racemes. Also in the second year, a significant increase in seed yield was obtained increasing water supply. The highest seed yield, was obtained by I100N120 (2023.6 kg ha^{-1}), and the lowest by I0N0 (230.5 kg ha^{-1}).

On the average of nitrogen fertilization levels, the total seed yield decreased by 34.4%, 48.2% and 66.8% when the water availability was reduced from the maximum restitution (I100) to I60, I30 and I0 treatments, respectively.

Koutroubas et al. (2000) reported that seed yield of irrigated castor was significantly higher than unirrigated control and concluded that irrigating castor with equivalent water between 75 and 100% of evapotranspiration was adequate to obtain higher economic yield under Mediterranean climate. Similar results have been reported by Laureti and Marras (1995).

The effect of nitrogen fertilization on total seed yield was less marked in I0 and I30 treatments as compared to the treatments with higher water availability that reached the highest seed yield. Indeed, the highest soil nitrogen content (N120) combined with high water availability (I60 and I100 treatments) was advantageous for castor productivity in terms of number of racemes per plant, capsules per raceme and seed weight. On the average of irrigation levels, the total seed yield decreased by 23.5% reducing the nitrogen fertilizer by 50% (N60) and by 39.4% without nitrogen fertilization (N0).

The contribution of each component to final seed yield was not stable across years. The highest yields for all I100 treatments in the first year were due to the higher number of capsules per raceme and higher seed weight as compared to other treatments. In the second year, despite the low seed weight, the higher number of racemes per plant determined an increase in seed yield for all treatments.

Arnaud (1990) reported that the highest yield obtained by irrigation supply was determined by an increased number of racemes and capsules per plant but not by the higher seed weight.

Oil content is dependent on genotype, environmental conditions, and their interactions (Singer et al., 2016) but is not influenced by N-fertilization, irrigation, planting time or plant spacing (Ramesh and Venkate, 2001; Souza et al., 2007; Diniz Neto et al., 2009; Soratto et al.,2011; Souza et al., 2012). Oil content in castor seeds is a trait with high heritability (Soratto et al., 2012). The oil content obtained in this study was in line with that reported by Anastasi et al. (2015) and Laureti et al. (1998) in castor cultivated in Mediterranean environment.

Seed oil content had marginal influence on oil yield, because it varied in a narrow range. According to Koutroubas et al. (1999), higher seed yields are usually obtained at the expense of the oil content, and there is a negative correlation between these two variables. In some studies, the seed oil content often has been found to increase in late maturing racemes (Fanan et al., 2009; Zuchi et al., 2010).

Many irrigation-based studies in castor revealed that oil content of seeds was not significantly affected by different irrigation regimes. However, oil yield increased with the maximum irrigation (Nagabhushanam and Raghavaiah, 2005), since oil yield mainly depended on the number of racemes per plant and on seed weight. As reported by Weiss (2000), N-fertilization seems to have minor effect on seed oil content. Some studies revealed that as nitrogen application increased, the percentage of oil decreased (Sawana et al., 2007; Farahani and Aref, 2008).

Higher soil water availability induced higher photosynthesis and transpiration rates, with consequently higher seed production.

The soil nitrogen availability did not affect both photosynthesis and transpiration rate.

As far water use efficiency is concerned, the results from the present study are in agreement with several researchers who reported that water use efficiency is generally higher under dry than well water conditions (Patel et al., 2004; Sesha and Bhaskar, 2005; Ramanjaneyulu et al., 2010).

4.5 Conclusions

Castor can grow as a semi-perennial plant in the Mediterranean environment, extending the growing season to a second year. In this way the crop may benefit of a suitable rainfall regime in fall-winter, thus reducing the irrigation requirements.

The results obtained in both years revealed that seed yield in castor is strongly depressed under severe water stressing conditions (no irrigation or minimum irrigation, e.g. 30% ETm restoration), while under moderate water deficit (e.g. irrigation at a 60% rate) it may achieve adequate levels. Of course, yield can be maximized with full irrigation (100% ETm restoration), when the availability of water for irrigation does not represent a limiting factor.

Irrigation is the main factor influencing number of racemes per plant, number of capsules per raceme, and seed weight. High soil nitrogen content combined with high water availability (I60 and I100 treatments) was advantageous for castor seed and oil yield.

The availability of irrigation water in semi-arid environments represents a rising problem in castor cultivation. Considering the results of this study, the highest seed and oil yields were obtained with the maximum irrigation and N- fertilization levels. However, the reduction of inputs to intermediate levels could be considered an environmental strategy with minimal yield losses.

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5 A Biorefinery model of Castor for the production of oil under different nitrogen fertilisation and biomethane with different biological pretreatments

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A Biorefinery model of Castor for the production of oil under different nitrogen fertilisation levels and biomethane with different biological pretreatments

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ABSTRACT: Castor (*Ricinus communis* L.) is an important worldwide oilseed crop whose inedible oil is widely used in industrial, pharmaceutical and agricultural sectors. Castor plants show a high conversion potential for use as biorefining feedstock.

The present study was conducted to investigate the effects of different nitrogen fertilization levels (0 and 120 kg ha^{-1}) on seed and oil yield and some agronomic traits.

In a biorefinery perspective, the residue biomass of seed processing was analyzed in term of fibrous composition and biomethane production carrying out a biological pretreatment using two white rot fungi (*Pleurotus ostreatus* and *Irpex lacteus*).

Nitrogen fertilization determined an increase on seed and oil yield and a difference on capsule husks composition. Fungal pretreatments of capsule husks showed promising effects on anaerobic digestion increasing biomethane yield as compared to untreated biomass.

The highest lignin degradation and the lowest cellulose loss during the pretreatment were obtained by *I. lacteus*, and this fungal pretreatment allowed to obtain the highest biomethane yield (103.2 Nml g^{-1} VS) for the fertilized biomass.

Keywords: *Ricinus communis* L; oil yield; seeds yield; anaerobic digestion; fungal pretreatment; Mediterranean environment

5.1 Introduction

Castor bean (*Ricinus communis* L.) is a non-edible multipurpose oilseed species of Euphorbiaceae family. It is indigenous to the Eastern Africa, most probably to Ethiopia, where the most wild and semi-cultivated types can be found (Scarpa and Guerci, 1982; Anjani, 2012) but nowadays it is developed worldwide from tropics to warm temperature regions.

Castor plant can be considered one of the most promising non-edible oil crops due to its high seed yield, its tolerance to a variety weather conditions and its ability to grow on marginal land and in semiarid climates (Falasca et al., 2012; Patanè et al., 2019). Castor is largely cultivated to use oil extracted from its seeds whose composition is made up of the following fatty acids: 91–95% ricinoleic acid, 4–5% linoleic acid and 1–2% palmitic and stearic acids (Severino and Auld, 2013a).

This composition makes the oil highly desirable for industrial and pharmaceutical uses such as the production of paints, coatings, inks, lubricants and cosmetics (Ogunniyi, 2006). Moreover, in the last few years, this non-edible vegetable oil has been investigated to produce biodiesel as an alternative to the edible oils traditionally used in biodiesel production, such as rapeseed, sunflower, soybean and palm (Dias et al., 2013; Keera et al., 2018).

Castor cultivation in the Mediterranean environment could be attractive for its capacity to grow in a variety of soils and climatic conditions reducing the competition with food crops and avoiding the "food vs. fuel" dispute (Abdul Hakim Shaah et al., 2021). Furthermore, Castor cultivation allows to valorize residual biomass with a biorefinery approach, since after the oil extraction from seeds for biodiesel production by transesterification, residual biomass, including stems, leaves and capsule husks, could be used for bioethanol or biogas production, significantly increasing the profitability of the crop (Ogunniyi, 2006; Bateni et al., 2014; Patel et al., 2016).

It is increasingly recognized the potential of plant-based materials, in particular lignocellulosic residue, as precious feedstocks for bioenergy production, to replace a significant fraction of fossil resources in order to reduce the dependence on fossil fuels and mitigate climate changes (Freitas et al., 2021).

Thus, effective utilization of lignocellulosic biomass as biofuels, e.g., bioethanol and biomethane, could satisfy the need for energy sources and provide solutions for environmental concerns.

Biomethane produced by anaerobic digestion (AD) is a great technique for the energetic valorization of different types of biomasses, including lignocellulosic residues.

However, lignocellulosic biomass presents a recalcitrant and highly lignified cell wall structure due to the linkage between cellulose, hemicellulose and lignin. The interaction of these components determine resistance to anaerobic degradation, limiting the enzymatic hydrolysis phase during anaerobic digestion process; thus, a pretreatment phase is essential to modify the biomass structure and improve biomethane yield.

Most investigated pretreatments, such as physical and thermo-chemical processes, are costly and can consume large amounts of energy. Compared to the other pretreatment methods, biological pretreatment is more environmentally friendly and has several advantages including low or no formation of harmful chemicals, low energy requirements operating under mild conditions, and no special equipment is required for the process (Singh, 2021; Wu et al., 2022). Biological pretreatments make use of wood-degrading microorganisms, including white-rot fungi, brown-rot fungi, soft-rot fungi, and bacteria, that through the action of extracellular enzymes are able to degrade lignin decreasing cellulose crystallinity and increasing accessible surface area.

White rot fungi (WRF), due to their superior ability to selectively degrade lignocellulose, can be widely used in many biotechnological sectors, including biofuel and biorefinery, and appear to be very promising in the biological pretreatment of lignocellulosic biomass feedstock.

This study aimed to maximize the potential of castor plant using a biorefinery that combines seed and oil yield for biodiesel production and the valorization of capsule husks through biomethane production.

Nitrogen is an essential nutrient required by plants and it is the most limiting nutrient in the soil. Thus, nitrogen fertilization is an important management practice to optimize crop growth and to obtain high yields (Jamil et al., 2001; Al-Thabet, 2006).

Nitrogen fertilization has a significant impact on biogas and methane production since influences both biomass yield and chemical composition of biomass.

The aims of this study were to evaluate the effects of two nitrogen fertilization treatments on castor production and on yield and biomethane production by anaerobic digestion after a biological pretreatment of capsule husks using two WRF (*Pleurotus ostreatus* and *Irpex lacteus*).

5.2 Material and Methods

5.2.1 Field experiment

The field experiments were conducted over the period 2020-2021 at the Experimental farm of the University of Catania, Italy (10 m a.s.l., 37°25’ N lat., 15° 03’ E long.) in a typical xerofluvent soil.

The soil characteristics were: clay 32.3%, sand 55.9%, silt 11.8%, organic matter 1.4%, pH 7.6, total N 0.2%, available P_2O_5 46.1 mg kg^{-1}, exchangeable K_2O 293.3 mg kg^{-1}.

The bulk density was 1.1 g cm^{-3}.The soil moisture contents at field capacity (at −0.03 MPa) and nominal wilting point (at −1.5

MPa) were 27 and 11 g H_2O 100 g^{-1} dry weight, respectively.

The soil of the experimental area was ploughed before sowing and fertilized with 70 kg ha^{-1} P_2O_5 (as single superphosphate).

Two nitrogen fertilization levels, N0 (0 kg N ha^{-1}) and N120 (120 kg N ha^{-1}), were studied.

The amount of nitrogen for each level was applied as followed: 35% before sowing (as ammonium sulphate), 45% before flowering (as ammonium nitrate) and 20% at the beginning of grain formation (as ammonium nitrate). Irrigation was provided by a drip irrigation system, by restoring 100% of maximum crop evapotraspiration (ETm).

Irrigation was scheduled when the sum of daily maximum crop evapotranspiration (ETm) corresponded to the volume, subtracting rainfall events from the calculation. The daily ETm was calculated according to:

$$ETm = E0 \times Kp \times Kc$$

where ETm is the maximum daily evapotranspiration (mm); E0 is the evaporation of class-A pan (mm); Kp is the pan coefficient, equal to 0.80 in semi-arid environment. Crop coefficients were determined by previous observation (Kc): 0.4 from emergence to the four leaf stage; 0.7 from the 4 leaf stage to flowering; 1.2 from beginning of flowering to complete capsule development of the first raceme and 0.55 from first raceme to complete capsule senescence.

The irrigation volume was calculated according to:

$$V = 0.66 \times (FC - WP) \times \phi \times D \times 10^3$$

where V = water amount (mm); 0.66 = readily available water not limiting for evapotranspiration; FC = soil water content at field capacity (27% of dry soil weight); WP = soil water content at wilting point (11% of dry soil weight); ϕ = bulk density (1.1 g cm^{-3}); and D = rooting depth (0.6 m).

During the growing cycle total 1800 m^3 ha^{-1} of irrigation water was supplied.

A randomized complete block design with three replications was used.

Each plot measured 68 m^2 (8 x 8.5 m) with 1.7 m row to row spacing and 1 m plant to plant distance (sowing density 0.58 plants per $1m^2$) and consisted of five rows.

The seeds used in this experiment was a local castor variety selected by Di3A from Tunisian genotype.

Sowing was manually carried out on the 24th of June 2020 by placing three seeds per hole at 3 to 4 cm depth; the plots were hand thinned to one plant per hill when the plants were at the four leaf stage.

Meteorological conditions and potential evapotranspiration (ET0) were continuously measured using a weather station connected to a data logger (Delta-T, WS-GP1 Compact) and a Class A evaporation pan (mm d^{-1}).

During the growing season, the date of occurrence of the main phenological stages of primary racemes (seedling emergence, anthesis and seed physiological maturity) were recorded. The date of harvests of primary racemes were 30 October and 8 November 2020 for unfertilized and fertilized plants, respectively.

The harvest dates of secondary racemes were 6 December 2020, 12 December 2020 and 28 February 2021 for unfertilized plants, and 7 January, 27 January and 17 March 2021 for fertilized plants, according to the different flowering period.

The three central rows of each plot were used for measurements and harvested.

At harvest, the number of primary and secondary racemes per plant, and the insertion height of the primary raceme (measured from the ground to the first raceme insertion point) were recorded. In the laboratory, the primary raceme length (from the apex up to the end of peduncle) was measured. The first and the secondary racemes were kept separated and weighed; the capsules of each raceme were counted and manually separated from peduncle, and seeds were separated from capsules to determine the number of capsules per raceme and the seed weight.

5.2.2 Characterization of Feedstock

Capsule husks were oven-dried at 65 °C, milled and used for chemical analysis and biological pretreatment.

The total solids (TS), volatile solids (VS), and chemical composition of capsule were determined before pretreatment.

TS content was measured by drying samples at 105 °C to constant weight and VS content was estimated by an incineration in muffle furnace at 550 °C during 5 h (Sluiter, 2008).

TS and VS measurements were realized in triplicate.

The hemicellulose, cellulose and lignin content were determined according to the Van Soest method (Van Soest et al., 1991). To determine the ashes, ADL residue was ignited in muffle furnace at 550 °C for 5 hours and lignin content was calculated as the difference between ADL and ash.

5.2.3 Oil extraction

The seeds were grinded to be subjected to the oil extraction using the GM200 blade mill (Retsch). The oil content was determined according to Randall method by the use of a quantitative solvent extractor SER 148/6 (Velp Scientifica) (Randall, 1974).

The extraction was performed by immersion the cellulose thimble containing 3 g of sample in the boiling n-Hexane solvent, followed by a rinsing phase and a recovery phase. Extraction vessels were placed in an oven at 100 °C for 30 minutes to eliminate any solvent residues. The analyses were performed in triplicate.

5.2.4 Inoculum preparation

The fungal strains used in this study (*Pleurotus ostreatus* (MUT00002977) and *Irpex lacteus* (MUT00005918)) were purchased from Mycotheca Universitatis Taurinensis (MUT) of the Department of Life Sciences and Systems Biology, University of Turin (Italy). Fungi were activated on Malt Extract Agar plates and incubated at 26 °C for 7 days.

The inoculum preparation was performed as previously described by Piccitto et al. (2022). The full substrate colonization occurred after four weeks from the incubation, and sterile capsule husks colonized with *Pleurotus ostreatus* and *Irpex lacteus* were used as inoculum for the subsequent fungal pretreatment experiments.

5.2.5 Fungal Pretreatments

Sterile capsule husks and inoculum (fungal-colonized capsules) were mixed and added to 0.5 L reactors. Fungal pretreatments were performed at 30% (dry weight basis) inoculum ratio.

Deionized water was added to reach 70% moisture content. Reactors were covered with cotton plugs and incubated at 26 °C for 30 days.

The subsamples were taken at predetermined periods (10, 20, and 30 days) to determine cellulose, hemicellulose, and lignin content.

The dry matter loss and degradation of cellulose, hemicellulose, and lignin during fungal pretreatment were calculated as a percentage of the initial dry weight and fiber fractions before fungal pretreatment.

5.2.6 Biochemical Methane Potential (BMP) tests

The BMP test was performed by an automatic methanogenic potential detection system (AMPTS II, Automatic Methane Potential Test System, Bioprocess Control AB, Sweden).

The experiment was conducted at mesophilic conditions (38±1°C) in reactors of 500 mL each continuously mixing, with an inoculum substrate ratio of 1:3 in terms of grams of VS. All tests were performed in triplicate. The inoculum was originally obtained from an anaerobic digester located in Sicily and maintained in a reactor in the laboratory. To remove large and undigested particles the inoculum was filtered through a 2 mm porosity sieve and then it was stabilized in an incubator at 38 °C for 5 days.

TS and VS were determined both for the organic substrate and the inoculum as reported above.

Each reactor was connected to a 80 mL trap bottle of 3 M sodium hydroxide solution used for absorbing CO_2 from the raw gas. The remaining gas after scrubbing passed to ultra-low gas flow meters which were connected to the data analytical and acquisition system. The BMP test was run for 40 days.

Additionally, blank samples, only containing inoculum, were incubated. The resulting methane production of the substrate was determined by subtracting methane production of the blank (inoculum) from the substrate sample (substrate + inoculum).

The final value of cumulative methane production at the end of the test was defined as the experimental BMP of the substrate.

5.2.7 Statistical analysis

Agronomic data were subjected to statistical analysis using ANOVA according to the randomized blocks with three replications. Biomass content of the hemicellulose, cellulose, ADL, ash and NDS, were analyzed by one-way ANOVA with fertilization as fixed effect.

The pretreatment in laboratory data were statistically analyzed using ANOVA with completely randomized factorial design with three factors (pretreatment, nitrogen fertilization and time of measurement) considering all fixed factors. The daily and cumulated biomethane of untreated and fungal pretreated capsule husks after 40-day incubation were analyzed by a two-way ANOVA with fungal pretreatment and fertilization as fixed effect.

The biomethane yield per hectare was statistically analyzed using a factorial completely randomized with two factors (pretreatment, nitrogen fertilization) considering all fixed factors.

Before conducting the ANOVA, the Bartlett's test was run to verify the assumption of homogeneity of variances. Mean separation was performed according to the LSD test ($P \leq 0.05$). The data were statistically analyzed using R software (4.2.0, R Core Team, 2013).

5.3 Results

5.3.1 Meteorological conditions

Weather conditions during the field experiment reflect those typical of a Mediterranean environment.

A total of 231.6 mm and 208.8 mm of rainfall were recorded before the sowing time (March- June 2020) and from the sowing to the first harvest time (June-October 2020), respectively.

The cumulative rainfall for the harvest season (October 2020-March 2021) was 345.8 mm (Fig.1).

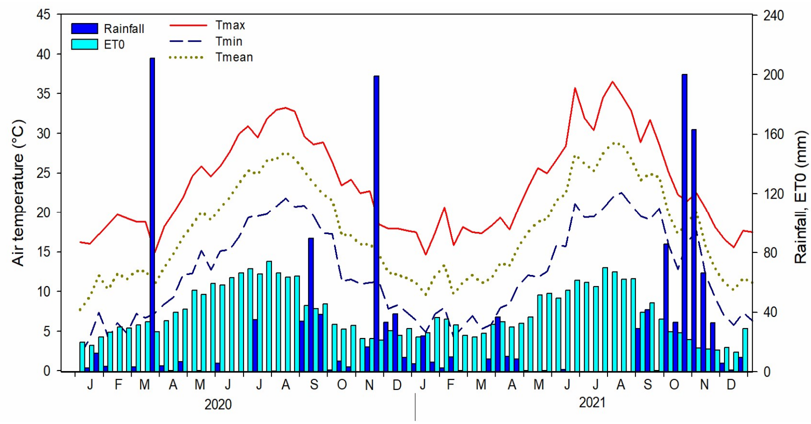

Figure 1. Meteorological trend (air temperature and rainfall) and reference evapotranspiration (ET0) during the 2-year study period (2020–2021) at the Experimental farm of the University of Catania, Italy (10 m a.s.l., 37°25' N lat., 15°03' E long.).

During the growing season, annual average temperatures were 22.9°C for the maximum, 11.9°C for the minimum and 17.2°C for the mean temperature. The reference evapotranspiration (ET0) was 1560.1 mm, with an average of 4.39 mm day^{-1}.

5.3.2 Phenological stages

Seedlings emerged after 7 days. The anthesis of primary raceme was 61 days after sowing for both nitrogen treatments. The physiological maturity of the first raceme was completed 128 days after sowing for the unfertilized treatment N0 and after 137 days for fertilized treatment N120 (Fig.2).

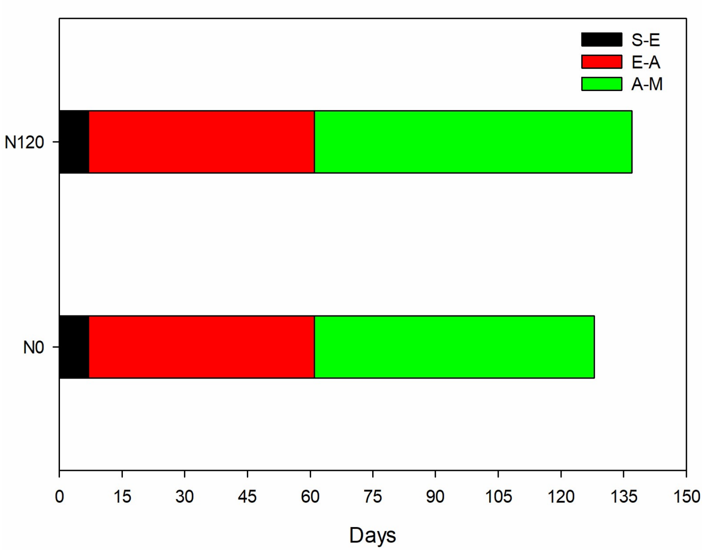

Figure 2. Phenological stage duration (days) from sowing to emergence (S-E), from emergence to anthesis (E-A), from anthesis to seed maturation (A-M) of primary raceme under fertilization levels.

5.3.3 Morphological characters, yield and yield components

Results regarding plant height up to primary raceme, length of primary raceme, number of secondary racemes, number of capsules per raceme, seed weight are presented in Table 1.

Table 1: Yield related components in Castor at different nitrogen fertilization.

Fertilization	Plant height up to primary raceme	Length of primary raceme (cm)	Number of secondary racemes	Number of capsules per raceme		Seed weight (g)	
				Primary	Secondary	Primary	Secondary
N0	$42.0^{a} \pm 2.1$	$28.3^{a} \pm 1.4$	$3.5^{b} \pm 0.2$	$59.0^{b} \pm 2.9$	$32.0^{b} \pm 1.6$	$0.2^{b} \pm 0.0$	$0.4^{a} \pm 0.0$
N120	$41.0^{a} \pm 2.1$	$26.0^{a} \pm 1.3$	$4.0^{a} \pm 0.2$	$66.2^{a} \pm 3.3$	$38.0^{a} \pm 1.9$	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.0$

Different letters in the same columns indicate significant differences between fertilization levels ($P \leq 0.05$); values are expressed as mean ± standard deviation (n = 3).

The analysis of variance (ANOVA) showed a significant fertilization main effect on number of secondary racemes, number of capsules per raceme for both primary and secondary racemes and seed weight of primary raceme.

Nitrogen fertilization had no significant effect on plant height up to primary raceme, length of primary raceme and seed weight of secondary racemes.

5.3.4 Seed yield and capsule husks yield (kg ha^{-1})

The analysis of variance (ANOVA) showed a significant fertilization main effect on primary, secondary and cumulative racemes yield.

The cumulative raceme yield was 2008 and 3620.1 kg ha^{-1} for N0 and N120, respectively (Table 2).

Table 2: One-way ANOVA for main effect (fertilization) on primary racemes yield, secondary racemes yield and total racemes yield. Degree of freedom (df) and adjusted mean square. Significance: p≤0.001 (***).

Source	df	Primary racemes yield	Secondary racemes yield	Cumulative yield
Fertilization	1	228462.11***	2239437.2***	3898460.8***
Error	4	905.16	9551.26	14630.47

The yield of primary and secondary racemes, composed by seeds and capsule husks for both fertilized treatments (N0 and N120) is reported in Fig. 3.

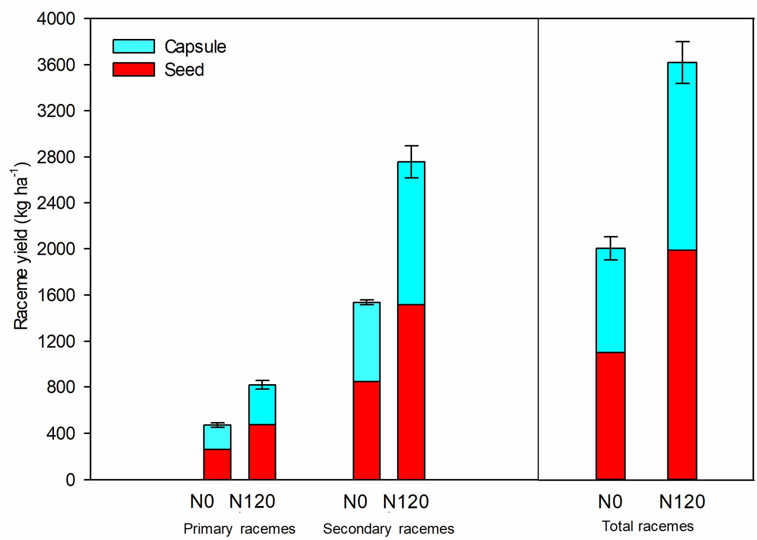

Figure 3. Castor yield components (kg ha^{-1} ±SE) of primary and secondary racemes under nitrogen fertilization levels (N0 - 0 kg N ha^{-1} and N120 - 120 kg N ha^{-1}). On the right, cumulative yield (kg ha^{-1}) of castor raceme under fertilized regimes (N0 and N120).

The seed yield for primary racemes was 258.9 and 473.5 kg ha^{-1} for N0 and N120, respectively, while for secondary one was 845.5 and 1517.6 kg ha^{-1} for unfertilized and fertilized treatment, respectively.

The capsule yield for primary racemes was 211.8 and 387.4 kg ha^{-1} for N0 and N120, respectively, while for secondary one was 691.8 and 1241.6 kg ha^{-1} for unfertilized and fertilized treatment, respectively.

The total seed and capsule husks yields were 1104.4 and 903.6 kg ha^{-1} for N0 and 1991.1 and 1629.1 kg ha^{-1} for N120, respectively.

5.3.5 Oil content and oil yield

The ANOVA showed a significant effect of raceme order on oil content. The oil yield was significantly influenced by fertilization and raceme order. Significant interactions "Fertilization x Raceme order" were also observed on oil yield (Table 3).

Table 3: Two-way ANOVA for fertilization (F) and raceme order (R) main effect and interaction (*FxR*) on oil content and oil yield of primary and secondary castor racemes. Degree of freedom (df) and adjusted mean square. Significance: p≤0.001 (***), not significant (ns).

Source	df	Oil content	Oil yield
Fertilization (F)	1	17.2^{ns}	77123.5***
Raceme order (R)	1	210.3***	434416.8***
FxR	1	3,7^{ns}	24061.1***
Error	8	3,6	637.3

The percentage of oil content in castor seeds was 35.8% and 34.5% for the primary raceme and 45.3% and 41.8% for the secondary racemes, for unfertilized and fertilized treatment, respectively. The average percentage of seed oil content was 43.1% and 40.1% for N0 and N120 treatment, respectively (Fig. 4).

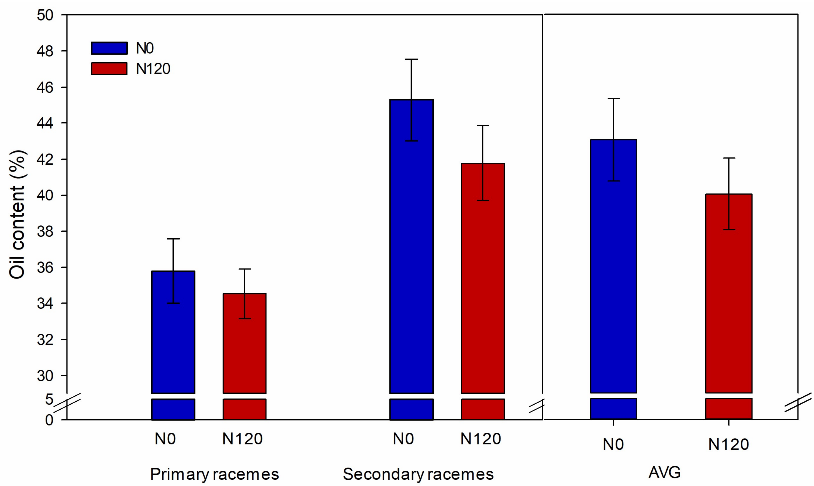

Figure 4. Seed oil content (% ±SE) of primary and secondary racemes under nitrogen fertilization levels (N0 - 0 kg N ha^{-1} and N120 - 120 kg N ha^{-1}). On the right, the average percentage of seed oil content under fertilized regimes (N0 and N120).

The oil yield for primary racemes was 92.6 and 163.4 kg ha^{-1} for N0 and N120 respectively. The oil yield for secondary racemes was 383.6 and 633.5 kg ha^{-1} for unfertilized and fertilized treatment, respectively.

The total oil yield was 476.1 and 796.8 kg ha^{-1} for N0 and N120, respectively (Fig. 5).

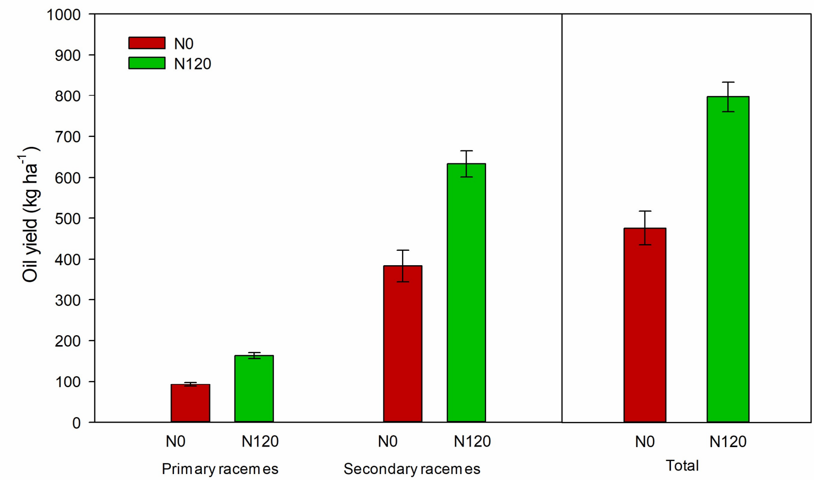

Figure 5. Oil yield (kg ha^{-1} ±SE) of primary and secondary racemes under nitrogen fertilization levels (N0 - 0 kg N ha^{-1} and N120 - 120 kg N ha^{-1}). On the right, cumulative oil yield (kg ha^{-1}) of castor racemes under fertilized regimes (N0 and N120). (LSD $_{FxR}$ p≤ 0.05) for Oil yield (47.53).

5.3.6 Capsule husks composition

The analysis of variance (ANOVA) of nitrogen fertilization as main effect on capsule husks composition is shown in Table 4.

Table 4: One-way ANOVA for main effect (fertilization) on hemicellulose content (H), cellulose content (C), lignin content (ADL), neutral detergent soluble content (NDS), ash content (ASH). Degree of freedom (df) and adjusted mean square. Significance: P≤0.05 (*), not significant (^{ns}).

Source	df	H	C	ADL	NDS	ASH
		%	%	%	%	%
Fertilization	1	1,2^{ns}	77,3*	9,44*	44,1^{ns}	0,032^{ns}
Error	4	3,2	6,7	0,92	28,1	0,0277

The effect of fertilization was significant on cellulose and lignin content, while hemicellulose, NDS and ash content did not differ.

NDS content was higher in the unfertilized (38.1 % w/w) as compared with fertilized treatment (32.7% w/w), while the content of hemicellulose and cellulose was higher in the fertilized residue (21.6 % and 36.5 % w/w, respectively) than unfertilized one (20.7 % and 29.3% w/w, respectively).

The unfertilized residue had a greater content of lignin (11.0 % w/w) and ash (0.8 % w/w) than the fertilized (8.5 % and 0.7 % w/w respectively) (Fig.6).

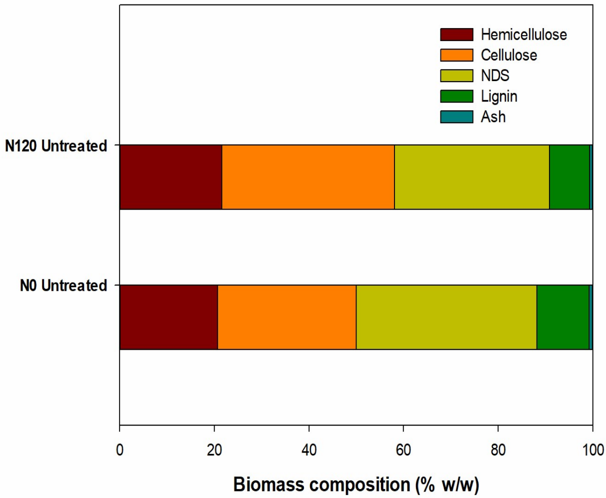

Figure 6. Biomass composition (% w/w) of capsule husks under nitrogen fertilization levels (N0 - 0 kg N ha^{-1} and N120 - 120 kg N ha^{-1}).

The ratio of structural carbohydrates (hemicellulose and cellulose) over lignin was determined in addition to the analysis of the various fractions of lignocellulose residue.

This measurement may be used to estimate the digestibility of the substrate being tested. For fertilized residue, the highest ratio was recorded (6.9).

5.3.7 Pretreatment effects on lignocellulosic biomass

The ANOVA showed a significant effect of pretreatment and fertilization on dry matter, cellulose and lignin degradation. Dry matter, hemicellulose, cellulose and lignin degradations were significantly influenced by time.

Significant interactions "Pretreatment x Time" and "Pretreatment x Fertilization x Time" were also observed on dry matter degradation and on lignin degradation, respectively (Table 5).

Table 5: ANOVA on degradation of capsule husks dry matter (DM), hemicellulose (H), cellulose (C) and acid detergent lignin (ADL) during 10, 20 and 30-day fungal pretreatment (*I. lacteus* and *P. ostreatus*) under fertilization levels. Degree of freedom (df) and adjusted mean square. Significance: P≤0.001 (***), P≤0.01 (**), P≤0.05 (*), not significant (^{ns}).

Source	df	DM	H	C	ADL
Pretreatment (P)	1	12.4^{*}	0.5^{ns}	55.5^{**}	49.1^{***}
Fertilization (F)	1	7.3^{*}	1.5^{ns}	778.6^{***}	22.7^{**}
Time (T)	2	843.1^{***}	$874.^{**}$	775.9^{***}	525.7^{***}
P x F	1	1.1^{ns}	14.4^{ns}	0.1^{ns}	10.2^{ns}
PxT	2	44.2^{***}	3.2^{ns}	8.1^{ns}	7.8^{ns}
FxT	2	3.3^{ns}	12.2^{ns}	8.1^{ns}	7.5^{ns}
PxFxT	2	1.3^{ns}	23.8^{ns}	13.3^{ns}	10.5^{*}
Error	24	1.6	10.2	6.7	2.7

The degradation of dry matter, cellulose, hemicellulose and lignin of capsules residue showed an increasing trend with time for both fungi (Fig. 7).

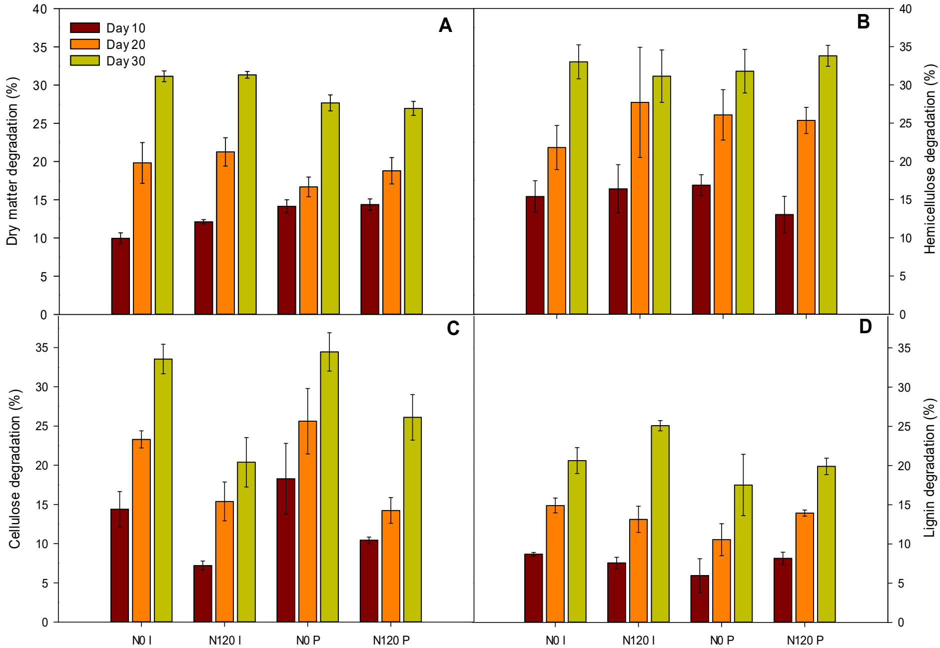

Figure 7. Degradation (% ±SE) of capsule husks components: (A) dry matter, (B) hemicellulose, (C) cellulose and (D) lignin during 10, 20 and 30-day fungal pretreatment in unfertilized and fertilized biomass (N0 - 0 kg N ha^{-1} and N120 - 120 kg N ha^{-1}) pretreated by *I. lacteus* (N0I and N120I, respectively) and *P. ostreatus* (N0P and N120P, respectively). Significant interaction (LSD $_{PxT}$ $P \leq 0.05$) for dry matter (3.14) and (LSD $_{PxFxT}$ $P \leq 0.05$) for ADL (11.38).

High percentage of degradation of dry matter was observed for *I. lacteus* for both unfertilized and fertilized substrate after 30 days of incubation (31.2% and 31.3%, respectively).

The percentages of degradation of dry matter for *P. ostreatus* were of 27.7% and 26.9% for N0 and N120 treatments (Fig. 7A).

For hemicellulose degradation, the highest loss was observed for *P. ostreatus* in fertilized treatment (33.8%) (Fig. 7B).

For cellulose, highest degradation was observed for *P. ostreatus* with a loss of 34.5% in unfertilized treatment (Fig. 7C).

The highest value of lignin loss was obtained by *I. lacteus* in fertilized sample (25.1%), followed by unfertilized biomass pretreated by *I. lacteus* (20.6%) (Fig. 7D).

5.3.8 Methane production

The ANOVA showed that daily biomethane production was significantly influenced by pretreatment, fertilization levels and incubation time (Table 6).

Table 6: ANOVA on daily biomethane (DCH4) of untreated and fungal pretreated capsule husks after 30-day incubation under two levels of nitrogen fertilization. Degree of freedom (df) and adjusted mean square significance: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*), not significant (^{ns}).

Source	df	DCH_4
Pretreatment (P)	2	$19,2^{**}$
Fertilization (F)	1	$15,8^{*}$
Time	1	$56,1^{***}$
P x F	2	$2,6^{ns}$
Error	473	2,9

The analysis of variance (ANOVA) of pretreatment and nitrogen fertilization as main effect on cumulated biomethane production ($\sum CH_4$) is shown in Table 7.

Table 7: Two-way ANOVA on cumulated biomethane ($\sum CH_4$) of untreated and fungal pretreated capsule husks after 30-day incubation under two levels of nitrogen fertilization. Degree of freedom (df) and adjusted mean square significance: $P \leq 0.001$ (***), $P \leq 0.05$ (*).

Source	df	∑CH4
Pretreatment (P)	2	1159.27***
Fertilization (F)	1	942.92***
P x F	2	154.18*
Error	12	32.81

Daily production (NmL g^{-1} VS d^{-1}) and cumulative methane production (NmL g^{-1} VS) during anaerobic digestion of untreated and fungal pretreated capsule husks are displayed in Fig. 8.

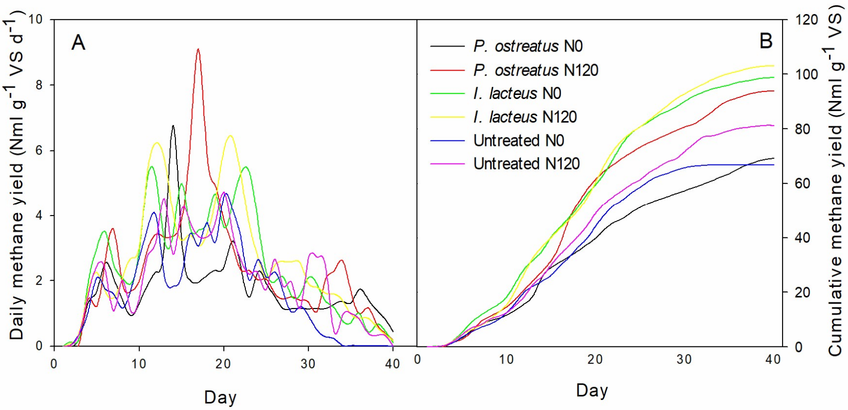

Figure 8. (A) Daily methane yield (Nml g^{-1} VS d^{-1}) and (B) cumulative methane yield (NmL g^{-1} VS) of capsule husks under fertilized regimes (N0 and N120) and fungal pretreatments (*I. lacteus* and *P. ostreatus*).

The daily biomethane production of capsules pretreated by *P. ostreatus* showed the highest peaks for fertilized treatment (9.1 Nml g^{-1} VS d^{-1}) after 17 days of digestion (Fig. 8A). Capsules pretreated by *I. lacteus* showed the maximum peak (6.4 Nml g^{-1} VS d^{-1}) on the 21^{st} day for fertilized biomass, while untreated capsule showed the peaks of daily methane production lower than the others treatments (4.6 and 4.7 Nml g^{-1} VS d^{-1} for N0 and N120, respectively) reached after 20 days.

Cumulative biomethane production was observed for 40 days until biomethane yield reached a plateau at the end of exponential phase (Fig. 8B, Table 8).

Table 8: Cumulative methane production in relation to different pretreatment and nitrogen level (N0 - 0 kg N ha^{-1} and N120 - 120 kg N ha^{-1}). Average values of all nitrogen levels (last column) and average values of all pretreatment (last row) followed by the same letter do not significantly differ at $p \leq 0.05$. Significant interaction ($LSD_{P x F}$ $P \leq 0.05$ (10.19)).

	Cumulative methane production (NmL g^{-1} VS)			
	Pleurotus ostreatus	*Irpex lacteus*	Untreated	AVG
N0	69.24	98.78	66.83	78.28 b
N120	93.89	103.16	81.23	92.76 a
AVG	81.56 b	100.97 a	74.03 c	

The initial lag phase lasted around three days until the complete adaptation of the bacterial flora to the lignocellulosic substrate.

The methane production obtained for the untreated capsules husks N0 and N120 was 66.8 NmL g^{-1} VS and 81.2 NmL g^{-1} VS, respectively.

P. ostreatus pretreated capsules achieved values of 69.2 Nml g^{-1} VS and 93.9 Nml g^{-1} VS for N0 and N120 fertilization respectively.

The methane yield reached by *I. lacteus* pretreated capsule husks was 98.8 Nml g^{-1} VS and 103.2 Nml g^{-1} VS for N0 and N120 fertilization levels, respectively.

Lignin, as is well known, has a negative impact on biomethane yield by significantly contributing to lignocellulose biomass recalcitrance.

The selectivity index, defined as lignin degradation over cellulose loss, is an important tool for assessing white rot fungi's ability to selectively degrade lignin.

Fig. 9 shows a correlation between the selectivity index for fungal pretreatment and the cumulative methane yield for *P. ostreatus* and *I. lacteus,* highlighting an increase in biomethane yield with a value of selectivity index higher than 0.5.

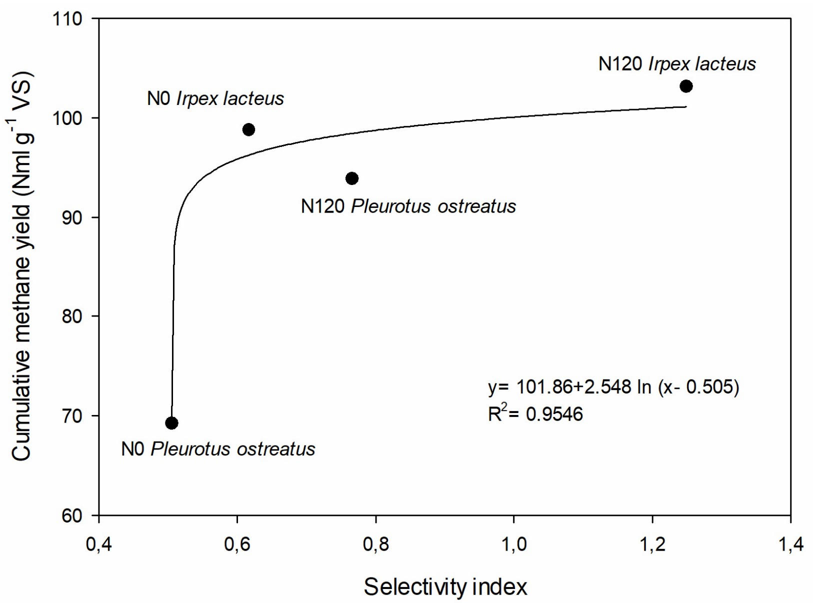

Figure 9. Non linear regression between the cumulative methane yield and selectivity index of *P. ostreatus* and *I. lacteus*.

5.3.9 Biomethane yield per hectare

The ANOVA revealed a significant effect of fertilization on biomethane yield (Table 9).

Table 9: Two-way ANOVA on biomethane yield of untreated and fungal pretreated capsule husks under two levels of nitrogen fertilization. Degree of freedom (df) and adjusted mean square significance: $P \leq 0.001$ (***), not significant (^{ns}).

Source	**df**	**Biomethane yield**
Pretreatment (P)	2	1210.5^{***}
Fertilization (F)	1	20922.4^{***}
PxF	2	88.1^{ns}
Error	12	57.8

The biomethane yield for untreated N0 and N120 biomass was 51.9 and 112.3 m^3 CH_4 ha^{-1}, respectively.

Among the fungal pretreated thesis, *I. lacteus* showed the highest biomethane yield per hectare (75.8 m^3 CH_4 ha^{-1} and 144.5 m^3 CH_4 ha^{-1} for N0 and N120, respectively) while *P. ostreatus* achieved values of 54.3 m^3 CH_4 ha^{-1} for N0 and 129.9 m^3 CH_4 ha^{-1} for N120 (Fig. 10).

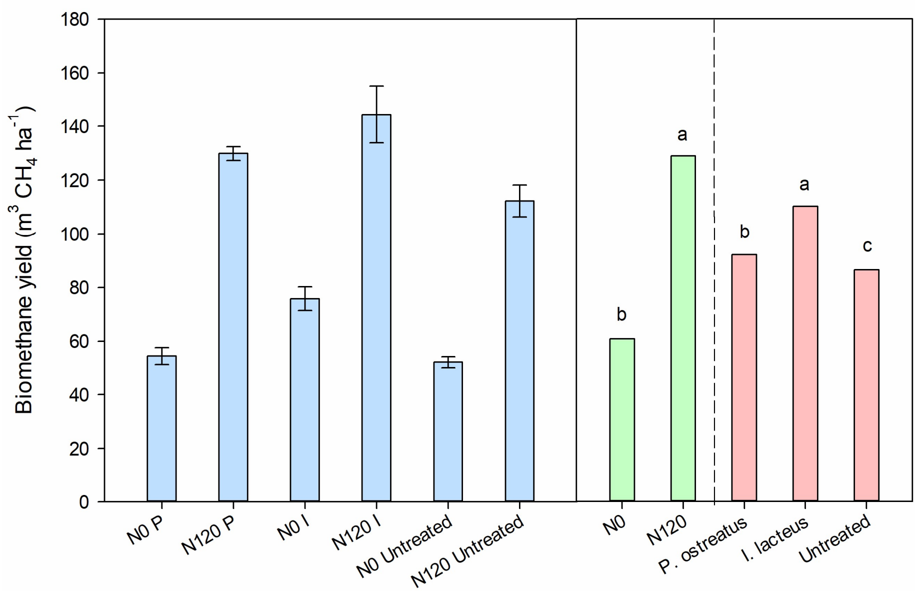

Figure 10. Biomethane yield (m^3 CH_4 ha^{-1} ±SE) of unfertilized and fertilized pretreated with *P. ostreatus* (N0P and N120P, respectively), unfertilized and fertilized pretreated with *I. lacteus* (N0I and N120I, respectively) and untreated unfertilized and fertilized capsule husks (N0 Untreated and N120 Untreated, respectively). On the right, mean separation, with different letters representing statistically significant means according to the LSD test ($P \leq 0.05$).

5.4 Discussion

Several studies reported a positive and significant correlations between the seed yield, and height at flowering, number of racemes per plant, raceme length, number of capsules per raceme, and 100 seed weight (Ramesh et al., 2001; Mullualem Atinafu et al., 2019).

Seed weight observed in the present study was in accordance with other studies, such as Goodarzi et al. (2015) who reported the 10 seed weight on primary raceme variable from 2.1 g to 3.3 g and Bhardwaj et al. (1996) who reported a 100-seed weight variable from 10 g to 44 g.

The increase of nitrogen dose significantly affected the main yield contributing traits, such as number of secondary racemes, number of capsules per raceme for both primary and secondary racemes and seed weight of primary raceme. These results are in line with Chatzakis et al. (2011) and Jamil et al. (2017).

Seed yield is considered the most important character for crop production improvement (Yousaf et al., 2018). The results showed that castor is able to obtain satisfactory seed yield, suggesting the adaptability to the Mediterranean environment as reported in a preliminary field experiment carried out by Anastasi et al. (2015) in southern Italy under rainfed regime to explore the feasibility of growing castor as semi-perennial plant.

The total seed yield was mainly affected by yield of secondary racemes with a percentage that ranged between 64 and 85% according to Severino et al. (2013b).

Koutroubas et al. (2000) suggested that the relative contribution of the racemes belonging to different order is not a stable characteristic, and depends on the environmental conditions, planting time, cultivar and cultivation techniques adopted.

Regarding the effect of nitrogen fertilization on castor seed yield, there was an increase in the treatment supplied with 120 kg N ha^{-1} for both primary and secondary seed yield and for cumulative seed yield. Pari et al. (2022) obtained similar results by castor hybrid under the Mediterranean climate with high-inputs field management (100 kg N per ha).

Seed oil content was lower for primary than secondary racemes; these results were in accordance with Souza et al. (2007), who reported a seed production from the secondary racemes with higher oil content, as compared to the primary racemes.

As reported by Weiss (2000), fertilization have a little effect on seed oil content. This is in accordance with our results, in fact the nitrogen fertilization treatment showed a lower seed oil content as compared to the unfertilized one and similar results were reported by previously studies carried on other oil crops. Farahbakhsh et al. (2005) observed a significantly decrease of oil percentage with an increase in nitrogen application on *Brassica napus* L. Sawana et al. (2007) reported that seed oil content was slightly decreased with an increase in the N rate from 95.2 to 142.8 kg ha^{-1} in cottonseed. In Froment et al. (2000) seed oil content in linseed decreased from 38.3% to 34.6% when additional N was applied.

Although seed oil content was reduced, seed oil yield was greater for N120 treatment than N0, since, as reported by Koutroubas et al. (1999), the main contribution to the total oil yield is given by the seed yield.

The composition analysis of capsule husks showed significant differences on cellulose and lignin content resulting from the different fertilization levels, with fertilized biomass containing more cellulose and less lignin than unfertilized biomass.

The presence of a significant amount of carbohydrates on capsule residue confirms the substrate's potential for use in anaerobic digestion for advanced biomethane production.

The ratio of structural carbohydrates (hemicellulose and cellulose) over lignin was used as indicator to estimate the digestibility of the substrate on anaerobic digestion process (Oleszek and Matyka, 2017).

The highest ratio was reported by the fertilized residue suggesting that nitrogen fertilization had a positive effect on lignocellulose susceptibility to anaerobic digestion.

However, the recalcitrant nature of capsule husks, caused by the high content of lignin, is an obstacle to their direct conversion. Among pretreatment methods, the biological pretreatment could play a key role in order to reduce the use of chemicals and energy inputs.

Several studies reported the positive effects of fungal pretreatment on degradation of lignocellulosic biomass (Wan and Li, 2010; Zheng et al., 2014; Kuijk et al., 2016; Noonari et al., 2020; Piccitto et al., 2022). *P. ostreatus* is one of the most studied white rot fungi able of producing a hydrolytic enzyme complex in different lignocellulosic biomass or under different fermentation conditions

(Elisashvili et al., 2008; Sánchez, 2009; Mustafa et al., 2016; Ding et al., 2019; Kainthola et al., 2019;).

P. ostreatus, on the other hand, is considered as a moderately selective lignin degrader, due to its significant consumption of cellulose in lignocellulosic substrates, especially with prolonged pretreatment time (Taniguchi et al., 2005). In our pretreatment experiments *P. ostreatus* showed the highest hemicellulose (33.8% for N120) and cellulose degradation (34.5% for N0), while *I. lacteus* reported the highest lignin loss (25.1% and 20.6% in fertilized and unfertilized treatment, respectively). The selective lignin-degrading capability of *I. lacteus* was also investigated by Yu et al. (2010) who reported a lignin loss from 75.67% to 80.00% after a fungal treatment with I. lacteus during mild alkaline pretreatment on corn stalks.

However, the efficiency of fungal pretreatment depends on biomass and fungal selectivity depends on adopted process conditions (fungal strain, time, temperatures) (Wan and Li, 2012; Hernández-Beltrán et al., 2019).

The high content of holocellulose lost during pretreatment determines a decrease in biomethane production.

Limited studies have been conducted on potential biomethane production from castor plant and castor seed cake but there is no research on biomethane production from capsule husks.

Bateni et al. (2014) evaluated the effect of alkaline pretreatment at different temperatures and time to improve biomethane yield from castor stem, leaves and castor seed cake. Pretreatment increased the biomethane production of castor stem from 80.8 to 145.5 mL g^{-1} VS.

In contrast, alkaline pretreatment had a negative effect on the biomethane production from both leaves and castor seed cake (Bateni et al., 2014).

In our experiments, *P. ostreatus* and *I. lacteus* pretreatment showed promising results for anaerobic digestion of capsule husks increasing biomethane yield as compared to untreated biomass.

I. lacteus pretreatment reached the highest biomethane yield (103.2 Nml g^{-1} VS) for the fertilized biomass thanks to the higher consumption of lignin (25.1%) and the lower cellulose loss (20.4%) that occurred during the pretreatment as compared to the other pretreatments. This is confirmed by the highest selectivity value (1.25) reached by *I. lacteus*.

Despite the consumption of cellulose that occurs during fungal growth, the pretreatment contributes to degradation of lignocellulosic biomass making cellulose and hemicellulose more accessible to microbial attack during anaerobic digestion and improving biomethane yield as compared to the untreated biomass.

The results highlighted the positive effect of nitrogen fertilization on biomethane production, due to the differences in composition between unfertilized and fertilized biomass. Fertilized biomass presented a greater content of hemicellulose and cellulose and a lower content of lignin than unfertilized one. Previous studies observed similar results on fertilized composition biomass of different lignocellulosic crops (Blümmel et al., 2003; Oleszek and Matyka, 2017; Scordia et al., 2020; Mahmood et al., 2022).

The positive effects of nitrogen fertilization and of fungal pretreatment were also found on biomethane yield per hectare.

5.5 Conclusions

This study highlighted the suitability of Castor, able to provide satisfactory seed and oil yield, to be cultivated in the Mediterranean climate. The analysis of composition allowed to consider castor capsule residue as a potential substrate useful for anaerobic digestion to produce advanced biomethane.

Nitrogen fertilized biomass showed a higher content of cellulose and a lower content of lignin than unfertilized biomass, and this composition determined the highest biomethane production.

However, the presence of lignin, is an obstacle for bioenergy conversion processes. Thus, a pretreatment is necessary to degrade lignin and improve the efficiency of conversion.

P. ostreatus and *I. lacteus*, two of the most common strain used on biological pretreatment, confirmed their capability to selectively degrade lignin with minimum cellulose loss improving biomethane yield.

Further studies are necessary to ascertain the optimal agronomic practices to improve seed and oil yield.

Moreover, in a biorefinery perspective, investigation of other pretreatment methods could help to enhance the energy conversion of this residual substrate and of other parts of the plant, such as stem and leaves.

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6 General conclusions and discussions

The present research project investigated the adaptability of Castor (*Ricinus communis* L.) in semi-arid Mediterranean environment and the bioenergetic potential of this crop evaluating the biomethane production from seed processing residuals. The project included two main research lines:

- The optimization of the cultivation techniques through the evaluation of different agronomical inputs;
- The improvement of the energy conversion process (biomethane production).

Regarding the first line, the results obtained from field trials revealed that Castor is a promising crop well adapted to grow in the southern Mediterranean basin. The main agronomical inputs investigated were water and fertilization requirements.

The castor field results showed in Chapter 2 relating to the establishment year and first harvest reported significant increases in seed yield with increased water supply. The yield increase in response to irrigation was associated with more racemes per plant, more capsules per plant on the first and other racemes and with higher seeds weight per capsule. Fertilization had a positive effect on primary racemes seed yield but with low water availability induced a delay in the development of secondary racemes, causing a reduction of mean seed yield from secondary racemes.

With a view to better study the castor local variety, it was compared to four dwarf hybrids to evaluate the response to different sowing date in terms of seed yield and growing cycle duration (Chapter 3). The results confirmed that the first sowing date (April) is the best for our local variety since allowed to satisfy the thermal requirements for germination and to complete the physiological maturity for both primary and secondary racemes obtaining high seed yield.

In Chapter 4, the possibility of exploiting the perennial habit of this species was investigated, keeping the crop over a two-year period. Sowing performed in April allowed maintaining Castor as a semiperennial, extending the crop cycle during a period of the year with a suitable rainfall regime, reducing irrigation requirements. Castor plants showed a tolerance ability under water stress conditions. The results of both experimental years showed that seed yield decreased under severe lack of water availability (I0 and I30 treatments), while under moderate water scarcity (I60) it was satisfactory. Yield increased by 50% with the maximum water supplied (I100 treatment). Irrigation is the main factor influencing number of racemes per plant, number of capsules per raceme, and seed weight. High soil nitrogen content combined with high water availability (I60 and I100 treatments) was advantageous for castor productivity.

The positive response of nitrogen fertilization was confirmed in Chapter 5, which reported the results of a field trial conducted investigating the effect of two fertilization levels with the maximum water availability. The maximum nitrogen level (120 kg N ha^{-1}) determined an increase on seed yield for both primary and secondary racemes since significantly affected the main yield contributing traits, such as number of secondary racemes, number of capsules per raceme for both primary and secondary racemes and seed weight of primary raceme.

Concerning the improvement of the energy conversion process, in Chapter 5, Castor was evaluated in terms of biomethane production from a residual lignocellulosic substrate. Castor plant has a high potential for use as a biorefining feedstock. The oil could be used for biodiesel production while other parts of the plant, including stem, seed cake, and leaves, which are among the lignocellulosic materials, could be used for biogas and ethanol production.

The results of capsule husks composition allowed to consider this substrate useful for anaerobic digestion to produce advanced biomethane and showed the differences resulted by the different fertilization levels, with a greater content of cellulose and a lower content of lignin on fertilized biomass as compared with the unfertilized. However, the presence of lignin is an obstacle for bioenergy conversion processes.

Thus, a pretreatment is necessary to degrade lignin and improve the efficiency of conversion. Fungal pretreatment carried out on capsule husks using *P. ostreatus* and *I. lacteus*, modified the composition of biomass selectively degrading lignin with minimum cellulose loss improving biomethane yield.

The results of the present thesis indicate that castor grows well under mild climate conditions of Mediterranean environment with sowing performed in April. The seed yield achieved in this study ranged between 2 and 4 kg ha^{-1} under mid-high input levels. The high bioenergetic potential of this crop was confirmed by its high oil content and by the great adaptability of residual biomass to anaerobic digestion process for advanced biomethane production.

Further studies are necessary to identify the optimal crop management (plant density, harvesting time and harvesting methods) to better understand the potential of this crop. Moreover, in a biorefinery perspective, investigation of other pretreatment methods could help to enhance the energy conversion of this residual substrate and of other parts of the plant, such as stem and leaves.

7 Annexes: Scientific Curriculum

7.1 Research and Professional experience

- 2019-2022: PhD course in Agricultural, Food and Environmental Science, University of Catania. Research project: “Castor oil as an innovative raw material for an energy supply chain in semi-arid environments”. Under the supervision of Prof. Salvatore Luciano Cosentino and Dr. Danilo Scordia.

- July-Dec 2021: Biomass Department of the Centre for Renewable Energy Sources and Saving (CRES), Pikermi, Greece. Research program: "The adaptation of Castor in semi-arid Mediterranean environment". Under the supervision of Dr. Eftymia Alexopoulou.

- Jan-Apr 2021–Jan-Feb 2022: EXCOSYSTEM SRL Company, Augusta, Italy. Evaluation of soil improver NIURA® to reduce irrigation input. Under the supervision of Dr. Salvatore Giamblanco.

- Dec 2017-Oct 2019: Scholarship at the Department of Agriculture, Food and Environment, University of Catania, supervisor Prof. Cosentino. Research program: "Evaluation of endemic grasses and biomass species for energy production suitable to the Mediterranean environment".

- Sep 2016 - Nov 2017: Scholarship at the Department of Agriculture, Food and Environment, University of Catania, supervisor Prof. Salvatore Luciano Cosentino. Research program: "Characterization of endemic grasses and new species suitable for Southern European environments", in the frame of the EU funded Project OPTIMA "Optimization of Perennial Grasses for Biomass Production".

- Mar 2015 - Aug 2016: Scholarship at the Department of Agriculture, Food and Environment, University of Catania, supervisor Prof. Salvatore Luciano Cosentino. Research program: "Characterization of endemic grasses and new species suitable for Southern European environments ", in the frame of the EU funded Project OPTIMA "Optimization of Perennial Grasses for Biomass Production".

7.2 *Education and Professional qualifications*

- Nov 2010: Qualification as agronomist and forestry doctor (senior)

- 2008-2009: Master's Degree in Agricultural Sciences and Technologies, University of Catania. Degree thesis: *"The structure stability of some volcanic soils of the Etna Park in the presence of holm-oak, oak and chestnut woods*".

- 1999: High School diploma: Liceo Classico – Istituto Superiore "Ven. Ignazio Capizzi", Bronte (CT).

7.3 Papers published in indexed peer-reviewed journals and in indexed proceedings of international conferences

- **Calcagno S**, Copani V, Castiglione R, Buscemi L, Piccitto A, Scordia D, Testa G, Cafaro V, Cosentino SL. The best castor Hybrids sowing date in order to escape adverse weather during start and final phases of growth cycle. In European Biomass Conference and Exhibition Proceedings; 2022; pp. 116-119. ISBN: 978-88-89407-22-6, DOI:10.5071/30thEUBCE2022-1AV.4.3.

- Piccitto A, **Calcagno S**, Corinzia S.A, Scordia D, Cosentino S.L., Castiglione R, Cafaro V, Testa G. Biomethane potentials from Castor Capsule Shells Pretreated with White-Rot Fungi. In European Biomass Conference and Exhibition Proceedings; 2022; pp. 769-772. ISBN: 978-88-89407-22-6, DOI:10.5071/30thEUBCE2022-4CV.3.9.

- **Calcagno S**, Piccitto A, Copani V, Corinzia SA, Scordia D, Testa G, Patanè C, Cosentino SL. Castor Oil as an Innovative Raw Material for an Energy Supply Chain in Semi-Arid Environments. In European Biomass Conference and Exhibition Proceedings; 2021; pp. 85–88. ISBN: 978-88-89407-21-9, DOI:10.5071/29thEUBCE2021-1BO.7.5.

- Piccitto A, **Calcagno S**, Copani V, Testa G, Scordia D, Patanè C, Cosentino SL. Oil Production of Diverse Mediterranean Castor Genotypes. In European Biomass Conference and Exhibition Proceedings; 2021; pp 362–365. ISBN:978-88-89407-21-9 DOI:10.5071/29thEUBCE2021-1DV.5.15.

- Piccitto A, Corinzia SA, Danilo Scordia, **Calcagno S**, Ciaramella BR, Patanè C, Cosentino SL, Testa G. Biomethane potential of an old plantation of giant reed clones with two irrigation levels. In European Biomass Conference and Exhibition Proceedings; 2021. pp 234 - 237. ISBN:978-88-89407-20-2, DOI:10.5071/28thEUBCE2020-1DV.2.29.

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- Patanè, C., Cosentino, S.L., **Calcagno S**., Pulvirenti, L., Siracusa, L. How do sowing time and plant density affect the pigments safflomins and carthamin in florets of safflower? (2020) Industrial Crops and Products, 148, 112313. DOI: 10.1016/j.indcrop.2020.112313.

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- Scordia D, **Calcagno S**, Piccitto A, Corinzia SA, Testa G, Ciaramella BR, Cosentino SL. Advanced Biomethane Production by Arundo Donax under Changing Harvest Time

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- Scordia D, Testa G, **Calcagno S**, Corinzia AS, Ciaramella BR, Piccitto A, Patanè C, Cosentino SL. Potential and Actual Yield of African Fodder Cane (Saccharum Spontaneum Ssp. Aegyptiacum) on Areas Affected by Biophysical Constraints. In European Biomass Conference and Exhibition Proceedings; 2020; pp. 34–40. ISBN:978-88-89407-20-2, DOI:10.5071/28thEUBCE2020-1BO.9.2.

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7.4 Proceedings of National Conference

- **Calcagno S**, Piccitto A, Copani V, Corinzia SA, Scordia D, Testa G, Patanè C, Cosentino SL. Effect of Different Irrigation Leves and Nitrogen Doses on Seed Yield of Castor. 2021. Proceedings of the 50th Conference of the Italian Society of Agronomy (Dalla Marta A., Maucieri C., Ventrella D., Eds.), Udine, Italy, 15th-17th September 2021, pag. 145-146. ISBN: 978-88-908499-4-7.

- Piccitto A, **Calcagno S**, Copani V, Testa G., Scordia D, Patanè C, Cosentino SL. Evaluation of diverse mediterranean castor genotypes. 2021. Proceedings of the 50th Conference of the Italian Society of Agronomy (Dalla Marta A., Maucieri C., Ventrella D., Eds.), Udine, Italy, 15th-17th September 2021, pag. 137-138. ISBN: 978-88-908499-4-7.

- Scordia D, Corinzia SA, Piccitto A, Ciaramella BR, **Calcagno S**, Testa G, Patanè C, Cosentino SL. First harvest results of reduced and well-watered perennial grass clones, species and hybrids. 2021. Proceedings of the 50th

Conference of the Italian Society of Agronomy (Dalla Marta A., Maucieri C., Ventrella D., Eds.), Udine, Italy, 15th-17th September 2021, pag. 149-150. ISBN: 978-88-908499-4-7.

- Corinzia SA, Ciaramella BR, **Calcagno S**, Scordia D, Piccitto A, Patanè C, Cosentino SL, Testa G. Evaluation of Monoecious and Dioecious Hemp Genotypes in Southern Italy. 2020. Proceedings of the 49th Conference of the Italian Society of Agronomy (Dalla Marta A., Ventrella D., Eds.), Bari, Italy, 16th -18th September 2020, pag.127-128. ISBN 978-88-908499-3-0.

- Corinzia SA, Scordia D, **Calcagno S**, Piccitto A, Testa G, Ciaramella BR, Cosentino SL. Leaf Gas Exchanges and Instantaneous Water Use Efficiency of Several Perennial Bioenergy Grasses. 2020. Proceedings of the 49th Conference of the Italian Society of Agronomy (Dalla Marta A., Ventrella D., Eds.), Bari, Italy, 16th -18th September 2020, pag.129-130. ISBN 978-88-908499-3-0.

- Ciaramella BR, Corinzia SA, Piccitto A, Scordia D, **Calcagno S**, Cosentino SL, Testa G. Adaptability of Industrial Hemp to Increasing Level of Heavy Metals in Soil. 2020. Proceedings of the 49th Conference of the Italian Society of Agronomy (Dalla Marta A., Ventrella D., Eds.), Bari, Italy, 16th -18th September 2020, pag.125-126. ISBN 978-88-908499-3-0.

- Scordia D, **Calcagno S**, Piccitto A, Corinzia SA, Testa G, Ciaramella BR, Cosentino SL. Harvest Time and Nitrogen Fertilization Effect on Advanced Biomethane Production by Giant Reed. 2020. Proceedings of the 49th Conference of the

Italian Society of Agronomy (Dalla Marta A., Ventrella D., Eds.), Bari, Italy, 16th -18th September 2020, pag.139-140. ISBN 978-88-908499-3-0.

7.5 *Attended Seminars / Courses / Meetings*

- Annual and technical meetings of the H2020 project "MAGIC" - Marginal lands for Growing Industrial Crops: Turning a burden into an opportunity (H2020-RUR-2016-2017).

- "Sviluppo di Induttori di Resistenza a Patogeni vascolari degli Agrumi" (University of Catania)

- "Le energie rinnovabili- eolico, solare e biomasse" (Leo 108YA-108YB - University of Catania)

- "EIT food innovator fellowship " (Impact Hub Siracusa, Dr. Notarbartolo)

- "Corso di Analisi Statistica Multivariata" (Prof. Corrado Dimauro e Alberto Cesarani, University of Sassari)

- "Biometry and data analysis- Statistica di base con applicazioni con il programma R" - Prof. Corrado Dimauro e Alberto Cesarani, Department of Agricultural, Food and environmental (University of Catania).