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RESEARCH ARTICLE

Alternative use of tobacco as a sustainable crop for seed oil, biofuel, and biomass

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Abstract Tobacco, *Nicotiana tabacum*, is an industrial crop traditionally used for manufacturing cigarettes. However, due to restriction of European subsidies, an alternative use of tobacco is needed, such as biofuel or biomass. Tobacco is indeed an oilseed crop with an oil yield ranging from 30 to 40 % of seed dry weight. Yet, there is still little information on the cultivation of tobacco for seed oil production. Here, we tested tobacco cultivation as an oilseed crop in Central Italy, where flue-cured tobacco is traditionally cultivated, with an additional trial in Northest Italy. We used the cultivar Solaris, selected for its reduced size and elevated number of flowers, and tested its adaptability to different field management practices in 2013–2014. Results show that in Central Italy fields, at the beginning of ripening, tobacco plants showed on the average a 94–105 cm height and a stem diameter between 1.3–1.6 cm; they had 123–151 capsules and each plant produced on average from 31 to 34 g of seeds. Seed production was evaluated to be 1.1–1.8 t/ha, with an oil yield up to 0.59 t/ha. In Northest Italy, two seed harvests determined a total seed yield of 4.5 t/ha, from which 1.48 t oil/ha could be obtained. The cultivar Solaris was extremely adaptable in terms of morphological parameters and seed yield to different management practices as well as climatic conditions. This study shows for the first time the cultivation feasibility in Italy of a small-size tobacco variety selected for high seed production. With a further optimization of the cultivation protocol to increase the oil yield and to use the by-products, tobacco can really become a novel industrial crop providing renewable sources for both biofuel and biomass as well.

Keywords Multiple stems · *Nicotiana tabacum* · "Solaris" · *Spodoptera* spp. · Tobacco flowers · Umbria region · Veneto region

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1 Introduction

The need for climate change adaptation and the raising concerns about energy security are the main reasons for the European Union (EU) to introduce the "20-20-20 targets" with the Renewable Energy Directive (2009/28/EC of 5 June 2009), which encourages the renewable energy development in the EU (Genovesi 2011). By 2020, the EU should be able to satisfy at least 20 % of its total energy needs with renewables, through national plans of each country, cutting off 20 % of both greenhouse gas emission and primary energy consumption. Furthermore, at least 10 % of transport fuels should come from renewable sources. The Italian national plan establishes that bioenergy should account for 45 % of the renewable energy while most part of this bioenergy (60 %) must be originated from solid biomass. The definition of bioenergy refers to the energy content in gaseous, liquid, and solid products derived from biological raw materials (biomass), which means biofuels for transport, substrates to generate electricity and heat, including biogas.

Biofuel for transport represents the major fraction of worldwide bioenergy production, and crop plants are one of the main sources for renewable raw material. Many biofuels are produced from food crops with high content of sugar or starch (e.g., corn for ethanol) and oil seeds (e.g., soybean for biodiesel) (first generation biofuels; see Ho et al. 2014 for review). However, the production sustainability has been questioned because of the competition with food crops and the impact on food supply and prices. Therefore, novel biofuel non-food and/or non-feed plant resources for bioenergy production need to be developed. Tobacco (*Nicotiana tabacum*) is an industrial crop traditionally used for cigarette and cigar manufacturing. However, applying cold pressing 93 % of the seed oil can be recovered (Stanisavljevic et al. 2009), and tobacco varieties can guarantee a good oil yield, from 30 to 40 % of seed dry weight, which has been successfully tested as a biodiesel (Giannelos et al. 2002; Usta 2005). Furthermore, the tobacco seed cake, which is an oil extraction by-product, turns out to be a suitable source of amino acids for animal feed formulations (Ouyang et al. 2003). In spite of being a promising non-food crop for bioenergy, the industrial use of tobacco oil has been hampered by the low seed production per hectare. Tobacco plants have also been genetically engineered to enhance their oil content in green tissues for potential biofuel production (Andrianov et al. 2010; Vanhercke et al. 2014). Despite this, due to EU restrictions on genetically modified plant cultivation, oil extraction from seeds may be a more reliable and sustainable approach than oil extraction from green tissues in tobacco.

Recently, the Sunchem Holding Company developed a cross-bred tobacco variety called "Solaris" (international patent PCT/IB/2007/053412). Solaris, compared with tobacco for smoking, contains no nicotine in the leaves and maximizes the production of flowers/seeds reducing leaf growth (Fogher 2008). Moreover, the re-sprouting from the stump after threshing makes multiple seed harvests (up to three times a year) possible for this variety in optimal environmental/agronomical conditions. By cold pressing, Solaris seeds produce 33 % of oil and the residual 67 % can be used for a protein cake (Fogher et al. 2011; Rossi et al. 2013). In addition, a significant quantity of capsules previously separated from seeds and the remaining green tissues (stems and leaves) in the field can be collected for biomass production.

The Italian total tobacco leaf production in 2014 was around 54,000 t, with a crop extension of 18,000 ha, ranking first in Europe (European Commission, Directorate General for Agriculture and Rural Development, Commission report, Raw tobacco production statistics, 2014–2003 harvests, http://ec.europa.eu/agriculture/tobacco/statistics/production-statistics_en.pdf). The Umbria region (Central Italy) accounts for about 30 % of the national production, mainly concentrated in the Perugia Province, playing a significant role in the local economy in terms of employment and income. In this province, 26.5 % of working hours carried out by agriculture seasonal workers in 2012 were dedicated to tobacco (Pantini et al. 2014). In the same year, the tobacco gross saleable production (GSP) in Umbria reached 3.6 million euro and 45.7 % of GSP was achieved in the municipality of Città di Castello (Perugia). In this city, tobacco cultivation and first transformation represented about 5 % of the local gross domestic product (GDP) and created an economic impact of 23.3 million euro in favor of local suppliers (Pantini et al. 2014). In view of the recent revision on the European tobacco policy against smoking and the significant tobacco cultivation impact on the environment, it is under discussion whether or not to continue this traditional cultivation in Umbria. First of all, farmers have developed tobacco cultivation skills and invested many economic resources on transformation facilities but now remain underutilized. Secondly, tobacco-cropping machinery producers have to deal with the market loss and eventually reconvert themselves to new mechanical productions as well. For all these reasons, using tobacco for other products could represent a valid alternative to reorganize a sustainable local economy. In this 2-year research, we tested the "Solaris" variety cultivation (Fig. 1) in the Umbria region, with an additional trial in the Veneto region (Northest Italy), using different field management practices to study its seed production performance in those arable lands traditionally used for flue-cured tobacco cultivation.

2 Materials and methods

2.1 Plant materials

Seeds of tobacco variety "Solaris" (owned by Sunchem Holding S.r.l., Arma di Taggia, Imperia, Italy) were sown in float trays under greenhouse conditions and, after an automated clipping step, transplanted in the field.

2.2 Site characterization and agronomic details

The field experiment was conducted in 2013 and 2014 in Marsciano (Perugia), Central Italy (Lat. 42° 54′ N, Long. 12° 20′ E, 151 m above sea level), one of the main flue-cured tobacco production areas in the Umbria region.

Another trial was conducted in 2014 in Pojana Maggiore (Vicenza), Northest Italy (Lat. 45° 17′ N, Long. 11° 30′ E, 14 m above sea level). Climatic condition data during the growing season in 2013 and 2014 was obtained from weather stations located near the experimental sites (Fig. 2).

In both years, the plant transplanting in Marsciano was delayed by adverse weather conditions. In 2013, two experimental fields were taken into consideration. In field 1 (1.3 ha, silty clay soil, the previous crop was wheat), tobacco was transplanted on May 27th. Some of the chemical properties of this field at 40 cm depth were sand 8.5 %, silt 51 %, clay 40.5 %, pH 8.5, cation exchange capacity 10.05 meq 100 g^{-1},

Fig. 1 Example of a field with tobacco cultivation for seed production. **a** Plants of cv. "Solaris" have inflorescences with open flowers. **b** Inflorescences at maturity with darkened capsules. It is possible to extract 33 % of oil per seed dry weight from these plants

organic matter 1.5 %, organic carbon 0.9 %, total nitrogen 0.14 %, assimilable phosphorus 57.3 mg kg^{-1}, and exchangeable potassium 207 mg kg^{-1}. Due to K and P abundance in the soil, a conventional binary N-P fertilizer (350 kg ha^{-1}), with a N:P ratio 34:16, was applied 4 weeks before transplanting because rainfall inhibited transplant immediately after fertilization application. After a tobacco transplant, since frequent showers prevented plants from normal growth, another nitrogen fertilization (ammonium nitrate) was applied, together with soil scratching for weed control, 6 weeks after transplanting at a uniform rate of 100 kg ha^{-1}. In total, 145 and 56 kg ha^{-1} of N and P were applied, respectively. In field 2

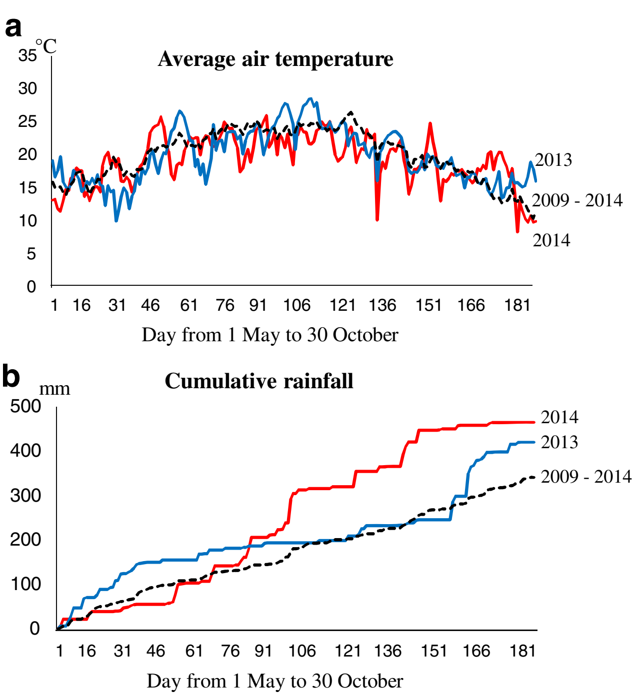

Fig. 2 Climatic conditions in Marsciano experimental fields during the growing seasons in 2013 and 2014. The average values from 2009 to 2014 are indicated with a *dotted line*. A 6-month period (from May 1st to October 30th) is taken into consideration for each year. **a** Time course of daily average air temperature (°C). **b** Daily values of cumulative precipitation in mm

(1.2 ha, clay soil, the previous crop was melon, transplantation date June 13th), a different fertilization system was adopted. The soil composition of this field is very similar to field 1, except for sand 4.8 %, silt 39 %, clay 56.2 %, and exchangeable potassium 160 mg kg^{-1}. As nitrogenous compounds of hen manure are easily mineralized to ammonia or nitrate, 1 week before transplanting, hen manure (900 kg ha^{-1}) with a N:P:K ratio 6:4:3 was applied and a combination of ammonium nitrate (50 kg ha^{-1}) plus ammonium dihydrogen phosphate (100 kg ha^{-1}) with a N:P total ratio (26 + 11):48 was applied 6 weeks after transplanting. Furthermore, 9 weeks after transplanting, a fertirrigation protocol (25 kg ha^{-1}, corresponding to a 12:20:24 N:P:K ratio) was implemented, with the addition of magnesium oxide and several microelements. In total, 81, 89, and 33 kg ha^{-1} of N, P, and K were applied, respectively. According to the climatic conditions and the irrigation systems, field 1 was irrigated by a sprinkler system with fixed irrigation volumes four times, with volumes of 360 m^3 ha^{-1} (36 mm) each irrigation, for a total irrigation amount of 144 mm, whereas field 2 was irrigated by droplet irrigation three times (the first time with a volume of 27.5 mm and the other two with 22.5 mm each) for a total irrigation amount of 72.5 mm. In 2014, tobacco "Solaris" was not cultivated in fields 1 and 2 in Marsciano, but the trial was performed in a different field of 1.8 ha (field 3, clay soil, the previous crop was maize) where plants were transplanted on June 5th. The composition of this soil is very similar to that of field 2. A conventional ternary N-P-K fertilizer (250 kg ha^{-1}), with a N:P:K ratio 8:18:28, was applied 4 weeks before transplanting together with 150 kg ha^{-1} of ammonium nitrate. Another nitrogen fertilization was applied as ammonium nitrate 3 weeks after transplanting at a uniform rate of 100 kg ha^{-1}. In total, 85, 45, and 70 kg ha^{-1} of N, P, and K were applied, respectively. Due to more than sufficient rainfall in July, water was delivered only three times in August by sprinkler irrigation for a total irrigation amount of 68 mm (Fig. 2b, day 62–92). Tobacco capsules were collected in September (in 2013) and in October (in 2014).

In 2014, an experimental field was also conducted in Pojana Maggiore. In field 4 (2.3 ha, sandy clay soil, flue-cured tobacco was the previous crop), tobacco was transplanted on May 30th. A fertilization program optimized by Sunchem Holding S.r.l. for "Solaris" cultivation in this area was adopted. Moreover, the timing of fertilizers was strongly influenced by weather conditions (rainy days). In detail, a conventional ternary fertilizer (180 kg ha^{-1}), with a N:P:K ratio 12:12:17, was applied 3 weeks before transplanting. Furthermore, starting from 3 weeks after transplanting, a fertirrigation protocol was applied with four fertilizations using conventional binary fertilizers, one in every 7–14 days. The first harvest was performed on August 20th. After the harvest, other two fertilizations (August 22th and September 2nd) by fertirrigation were implemented to guarantee a second harvest at the end of October. In total, 130, 127, and 138 kg ha^{-1} of N, P, and K were applied, respectively (98, 89, and 104 kg ha^{-1} of N, P, and K were applied before the 1st harvest). Field 4 was irrigated with fixed irrigation volumes by droplet irrigation (15 mm) six times for a total irrigation amount of 90 mm.

In all fields, a weed chemical control (pendimethalin or gliphosate) was applied a few days before transplanting. Since we were interested in tobacco seed production and sucker control inhibit the development of axillary buds and secondary inflorescences, no chemical application for sucker control was performed. In all fields, an infestation due to an unidentified *Spodoptera* species occurred (although it is very likely that the species was *Spodoptera litura*); thus, chemical treatments to control *Spodoptera* spp. (Lannate and Meteor) were applied in August and September.

2.3 Experimental design

Seedlings were transplanted in Marsciano fields in 2013 following a 1.15 m (between rows) × 0.16 m (between plants) pattern, corresponding to a final density of approximately 52,000 seedlings per hectare. In 2014, a minor density was applied (36,500 seedlings per hectare, 1.10 m between rows × 0.25 m between plants). To evaluate the morphological characteristics of "Solaris" in Marsciano, a small plot constituted of ten adjacent plants on the same row, with five replications per field, was set up in fields 1 and 2. For field 3, which was larger than the first two, six replications were prepared. In order to account for any variation in the field, each replication was located in a different area on a random sampling basis. In total, 50 plants were analyzed in fields 1 and 2 and 60 in field 3. Starting from the 42th day after transplanting, the following data were collected in each plant four times through the growing season: (1) plant height including inflorescences, (2) diameter of the main stem, (3) number of stems, and (4) developmental stage using the CORESTA Guide (2009), according to the Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) scale (Meier 2001). When seedlings were transplanted in field 4 in Pojana Maggiore, a 1.15 m (between rows) × 0.25 m (between plants) pattern was followed, corresponding to a final density of approximately 36,000 seedlings per hectare. No plot had been designed on this field. The average number of capsules per plant and seed dry weight was determined on 100 plants randomly chosen in the field.

Since no specific threshing machine for tobacco seeds collection without huge seed losses was available, in order to evaluate the seed yield in Marsciano fields, inflorescences were manually cut from each plant of the small plots (50 plants in fields 1 and 2 and 60 plants in field 3) and seed number and weight were assessed for each single plant, including the number of healthy or infested (by *Spodoptera* spp.) capsules. In addition, to obtain a more reliable value of seed yield in field condition, another large plot of 60 m^2 located approximately in the center of each field with 350 plants was taken into consideration. In this large plot, the seed yield per plant parameter was not calculated for each single plant as done for the small plots; instead, the total seed production of the large plots was divided by their number of plants. In the Pojana Maggiore experimental site, the capsules were manually cut from all the plants cultivated in the 2.3-ha field.

In all fields, when 20–30 % of the capsules turned from green to brown, the inflorescences were cut and left in site for 10–14 days to complete maturation. Seeds with a humidity value between 7.5–5.5 % of dry weight were then collected. They were put in big paper envelopes or other suitable containers to be dried to around 5 % of humidity value and then weighed.

In order to calculate the percentage of seed loss per plant due to *Spodoptera* spp. infestation at the Marsciano experimental site, the following formula for each plant of the small plots was applied:

$$\{1-(x\cdot a + y\cdot b)/[x\cdot(a+b)]\}\cdot 100$$

where x is the average weight of seeds per healthy capsules, y is the average weight of seeds per infested capsules, a is the number of healthy capsules, and b is the number of infested capsules. The x value is estimated by weighing seeds from 100 healthy capsules collected from plants of all the 5–6 small plot replications in each field. In the same way, the y value is estimated by weighing seeds from 100 infested capsules collected as described above.

2.4 Statistical analysis

The aim of our 2-year study in Marsciano (Central Italy) was to investigate the growth and seed yield of tobacco variety "Solaris" in different "environments", where "environment" stands for the total of all the variables (climate, soil, agronomic factors, plant density, etc.). Therefore, we did not consider the effect of every single treatment (e.g., different fertilization programs) instead we verified the "Solaris" adaptability, by measuring morphological parameters and seed yield, to different management practices and soil/climatic conditions. As concern to the morphological traits, we calculated their mean values on 50 (or 60 in the case of field 3) plants of the small replicated plots. This calculation was repeated four times for each field using the data recorded during the growing season (in Fig. 3, data are collected on the 75th day after transplant in fields 1, 2, and 3 is shown, while in Table 1, there are also data collected on the 92th and 91th day after transplant in fields 1 and 3, respectively). The yield parameters (shown in Tables 1 and 2) were recorded after the inflorescence harvest on the same plants analyzed for the morphological traits. The statistical significance of mean values of morphological and productive parameters was evaluated by Kruskal-Wallis one-way analysis of variance, followed by Dunn's post test, using the statistical software Graphpad InStat® version 3.00 for Windows 95 (Graphpad Software, San Diego, CA, USA, www.graphpad.com). Field 4 located in Pojana Maggiore (North East Italy) was not included in the ANOVA analysis because no plot had been prepared for this purpose.

3 Results and discussion

3.1 Field management practices and climatic conditions

To test the field performance of "Solaris" seed yield under different field management practices, two experimental fields were conducted in 2013 in Marsciano (fields 1 and 2) and two in 2014, one in Marsciano and one in Pojana Maggiore (fields 3 and 4, respectively). Pojana Maggiore is located in the Veneto region, Northest Italy, and we used this trial conducted in 2014 as a positive control for multiple harvests because, according to Sunchem internal reports, it was possible to obtain two seed harvests in the same growing season in Veneto. Indeed, multiple harvests would also be possible in Marsciano (Umbria region) if growing seasons were characterized by favorable climatic conditions. Field 1 was characterized by sprinkler irrigation (total water quantity 144 mm) and the use of chemical fertilizers with a conventional binary N and P fertilization, whereas field 2 was irrigated by droplet irrigation (total water quantity 72.5 mm) and fertilized mainly with hen manure plus fertirrigation. A conventional ternary N-P-K fertilization and sprinkler irrigation (total water quantity 68 mm) were utilized in field 3, while field 4 was fertirrigated. It is worth reporting that "Solaris" plant cultivation needs much less water than flue-cured tobacco cultivars. For example, in 2013, Virginia Bright tobacco fields in Marsciano area were watered by droplet irrigation up to volumes of 380–400 mm. Similar values (340–380 mm) were reported for drip irrigated Burley-type tobacco cultivated in Southern Italy (Campania region) during 1996–1997 (Sifola and Postiglione 2002).

The weather conditions in Marsciano during the growing seasons were similar in both 2013 and 2014 regarding daily average temperature (with few exceptions, for example, the second half of May) and precipitations amounts but not for rain distribution (Fig. 2). In 2013, rainfall was mainly concentrated in May and October; conversely, precipitation was more evenly distributed in 2014 (Fig. 2b), except for an impressive hailstorm occurred on June 15th followed by days of heavy rain which caused a partial flooding in the experimental field. Therefore, plant growth and development were seriously delayed. Comparing the daily average temperature and the rainfall amounts in these 2 years to the data from 2009 to 2014, the average daily temperatures of 2013 and 2014 were clearly lower than the average values of the 2009–2014 period during the first 2 weeks of June and from July 15th to August 15th (Fig. 2a). In addition, the amount of rain in this 2-year study turned out to be larger than the 2009–2014 period (Fig. 2b). Therefore, the climatic conditions in Marsciano failed to be very favorable in 2013 and 2014 for tobacco growth. The weather conditions of 2014 in Pojana Maggiore were characterized by rainy July (230 mm) and August (68 mm), with low daily average temperatures (22.8 in July and 22.3 °C in August).

3.2 "Solaris" morphological characters

Despite the different management practices and climatic conditions, the morphological characters (measured on plants located in the small replicated plots and reported as mean values in Fig. 3 and Table 1) of the tobacco plants were very similar during the 2 years of experimentation in Marsciano. On the 75th day after transplanting (DAT), plants in fields 1 and 3 had the same height and main stem diameter, but those in field 2 turned out to be higher, with a larger main stem and in an advanced phenological growing stage reaching an average BBCH value of 80, which corresponds to the beginning of ripening (Fig. 3). However, these few differences disappeared when plants at the same phenological stage were compared. Indeed, when plants in fields 1 and 3 also reached the beginning of ripening at 92 and 91 DAT, respectively, no significant difference was detected among plants in different fields (Table 1). At this stage, all plants showed an average height from 94 to 105 cm and an average main stem diameter comprised between 1.3 and 1.6 cm. Flue-cured tobacco plants, even if topped, are taller than "Solaris" plants. For example, the Virginia Bright tobacco in a period between 71 to 85 DAT can grow up to 109–188 cm (Orlando et al. 2011), and plant height at harvest of Burley type tobacco is comprised between 120 and 150 cm (Sifola and Postiglione 2002). These values indicate that tobacco variety "Solaris" plants expressed uniform vegetative traits and are smaller than other varieties commonly cultivated for leaf production. However, in the Pojana

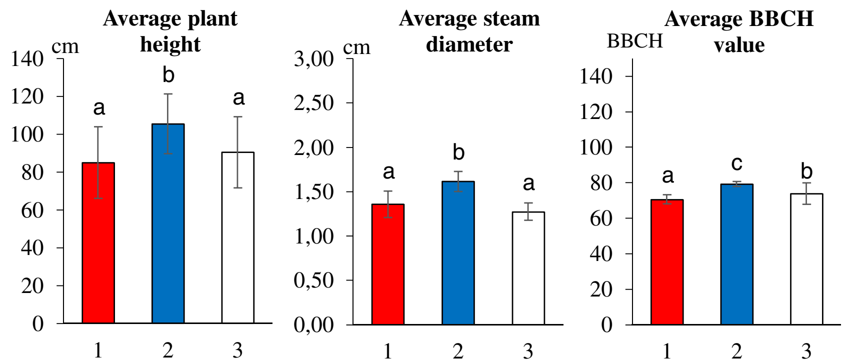

Fig. 3 Morphological traits of the tobacco plants measured on the 75th day after transplant in Marsciano. *Numbers* under the graphs indicate different experimental fields (fields 1 and 2 in 2013, field 3 in 2014). The *bars* show mean values of plant height including inflorescences, main stem diameter, and development stage according to the growth stages of the BBCH scale. This scale describes uniformly coding phenologically similar growth stages of plants (Meier 2001) and lays the basis for the CORESTA guide (2009). *Error bars* represent SD. *Different letters* indicate significant differences between fields as determined using analysis of variance (Dunn's post test, $P < 0.001$)

Maggiore field trial at the beginning of ripening, the height of "Solaris" plants ranged from 130 to 170 cm.

3.3 Seed production

"Solaris" plants at harvest in the Marsciano experimental site had an average number of 123–151 capsules from more than one stem and the seed yield of each plant ranged between 31 and 34 g (Table 1). In Pojana Maggiore site, the abovementioned values turned out to be higher than in Marsciano: on average 215 capsules and 62.5 g of seed yield per plant. In both sites, the dry weight of 1000 seeds was around 0.09 g, corresponding to the standard weight of "Solaris" seeds characterized by a low interspecific variability, with the minimum value at 0.07 and the maximum value at 0.10 g. Conversely, other authors reported an average weight of 0.07 g for 1000 seeds in an open field (Bucciarelli et al. 2012). The "per plant seed yield parameter" was initially calculated in each field in Marsciano using the plant inflorescences harvested both in the 5–6 small replicated plots (50–60 plants in total) and in the 60-m^2 plot located approximately in the center of the field (other 350 plants), as described in materials and methods. The average values obtained from the small plots (50–60 plants) and from the central plot (350 plants) were very similar with only a difference of 1–3 %. For example, in field 1, the average seed yield/plant value was 34.0 g for the small plots and 34.8 g for the central plot. Therefore, we reported in Table 1 the average value calculated only from the small plots (50–60 plants) analyzed in each field. By using the per plant seed yield parameter, the seed production was estimated from 1.1 to 1.8 t/ha (Table 1), a productivity significantly higher than that reported for flue-cured tobacco varieties (about 0.6–1.1 t seeds per hectare, Patel et al. 1998; Usta 2005). Considering the seed yield calculated above and the percentage (33 %) of extractable oil from seeds, the "Solaris" cultivar can produce up to 0.59 t of oil per hectare under the Central Italy environmental conditions and with the agricultural practices used in this experimentation.

Table 1 Morphological traits measured in the Marsciano experimental site when the plant growth was completed and post-harvest parameters

Field[1]	Morphological traits at BBCH value 80[2]					Yield parameters		
	No of stems/plant	Stem diameter (cm)	Height (cm)	DAT	Dry weight of 1000 seeds (g)	No of capsules/plant[3]	Seed yield (g)/plant	Yield (t)/ha[4]
1	1.04 ± 0.20^a	1.5 ± 0.39	94 ± 22.9	92	0.089 ± 0.006^{ab}	149 ± 83.09	34 ± 22.85	1.8
2	1.71 ± 0.58^b	1.6 ± 0.23	105 ± 15.8	75	0.087 ± 0.005^a	151 ± 62.92	32 ± 15.52	1.7
3	1.84 ± 0.59^b	1.3 ± 0.18	99 ± 16.3	91	0.094 ± 0.003^b	123 ± 64.40	31 ± 17.93	1.1

Different letters indicate significant ($P < 0.001$) differences

[1] Field numbers indicate different experimental fields (fields 1 and 2 in 2013, field 3 in 2014)

[2] The plants were at the beginning of ripening and this growth stage was classified as BBCH value 80 according to the CORESTA guide (2009), based on a BBCH scale for uniformly coding phenologically similar growth stages of plants (Meier 2001). Data were obtained from the tobacco plants located in the small replicated plots and reported as mean values ± SD. The days after transplanting (DAT) and yield (t)/ha are not reported as mean values

[3] This is the total number of capsules counted on different stems of each plant

[4] The yield in (t)/ha is calculated from a single harvest as follows: [average seed yield (g)/plant] × [no. of plants (52.000)/ha], except for field 3 where the number of plants was 36.500/ha

Table 2 Evaluation of the tobacco seed production damage due to *Spodoptera* spp. infestation

Field	% infested capsules	Seed loss (%)/plant
1	$16^{a} \pm 12.19$	11 ± 8.17
2	$24^{b} \pm 14.53$	15 ± 10.17
3	$21^{ab} \pm 15.02$	12 ± 7.94

Data were obtained from the tobacco plants located in the plots and reported as mean values ± SD. Field numbers indicate different experimental fields (fields 1 and 2 in 2013, field 3 in 2014). Different letters indicate significant ($P < 0.01$) differences

As it stands, such yield results competitive with soybean oil production in the USA. (0.52 t/ha, Balat and Balat 2010). Tobacco plants development failed to allow a second seed harvest in Marsciano trials, especially in 2014, when plants strongly impaired in development (due to adverse climatic conditions) produced only 1.1 t/ha of seeds. Conversely, the first seed harvest in August in Pojana Maggiore experimental site produced 2.1 t/ha, and the second in October reached 2.4 t/ha (total seed yield 4.5 t/ha, i.e., 1.48 t of extractable oil per hectare). Furthermore, an unexpected *Spodoptera* spp. infestation in Marsciano caused a seed loss of about 11–15 % per plant (Table 2); therefore, a more appropriate pest control can surely increase "Solaris" tobacco seed production. In 2010, from an experimental field located in the Abruzzo Region (Central Italy), Bucciarelli et al. (2012) reported a yield of 1.8 t/ha of seeds and an oil yield by cold pressing of 0.59 t/ha for a flue-cured tobacco line of the Kentucky type. This presumes (under the environmental conditions of Central Italy) a plateau value of 1.8–2.0 t/ha of seed yield could be expected for each single harvest. On the other hand, the small-size tobacco line "Solaris" requires less water and is less susceptible to lodging than flue-cured tobacco cultivars. The results reported here represent a preliminary step towards the use of tobacco seeds as a new industrial crop product, although in Central Italy, it is possible to obtain an average oil yield per area of 1.51 t/ha from a sunflower (Del Gatto and Di Candilo 2012). Hence, in this area, tobacco would be a valid alternative to sunflower for biofuel production if both the "Solaris" production protocol and the by-products utilization were optimized in the future. In order to establish a sustainable tobacco production chain for bioenergy, it is essential to improve "Solaris" seed production and use the residual biomass to produce biogas. Concerning seed production, nitrogen fertilization seems to be very important to guarantee healthy tobacco plants with many flowers and seeds, but to reduce the chemical products, organic fertilizers like hen manure can be administered to the soil before transplanting. Moreover, N fertilization is also important to support a fast plant growth, which can allow, if the climatic conditions were favorable, a first seed harvest in August and a second one in October. However, the role of N, as well as P and K, as possible limiting factor for "Solaris" seed production should be further investigated. In order to obtain at least two seed harvests, in addition to early transplant of young tobacco plants in field, one possible way is to set the first harvest 10–14 days in advance, cutting the inflorescences when only 5–10 % or even less of the capsules turn brown. Another key factor to make tobacco become a dedicated energy crop is the utilization of the residual biomass, which should be co-digested mixing with animal manure, and the biogas obtained can be burnt in an internal combustion heat and power generator. Indeed, this kind of bioenergy chains, as described in the paragraph below, is now strongly supported by Italian legislation.

3.4 Paving the way for sustainable bioenergy in Italy

Bioenergies are becoming more and more important in Europe as a renewable component of energy sources (Cherubini and Strømman 2011; Gabrielle et al. 2014). However, bioenergy chain sustainability is still controversial and requires further studies (Blengini et al. 2011; Panepinto et al. 2015). In Italy, a decree known as Burden Sharing established a distribution of efforts among various regions to achieve the national target (Ministerial Decree 2012). Umbria is committed to obtain 13.7 % of its energy from renewable sources within 2020, so it is necessary to develop sustainable bioenergy chains like the one suggested in this study with tobacco. The Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) has recently released a bioenergy plan, which describes a general strategy and identifies intervention priorities and operative instruments for promoting and disseminating bioenergy technologies (MiPAAF 2014). This policy, which also involves financial incentives, has stimulated the expansion in many regions of medium- and small-size systems of electric power and heat production from plant biomass combustion or gasification. According to the bioenergy plan guidelines, local sustainable bioenergy chains must be created primarily from crop residues and from no-food plant species turned into dedicated energy crops, with a particular focus on biomass utilization to generate electricity, heat, and biogas. Furthermore, it is necessary to integrate these energy crops into cropping systems with other crops grown for food and feed production, and particular attention must be paid to the chemical fertilizer reduction. It has been calculated that the only use of crop residues for energy production can generate a 5 % increase of the Italian agricultural GDP, with a beneficial economic impact on farmers (MiPAAF 2014).

4 Conclusion

To our knowledge, this is the first scientific report on the cultivation in Italy of a small-size tobacco variety selected for seed production. The "Solaris" cultivar is extremely adaptable in terms of morphological parameters and seed yield under different management practices and climatic conditions. Furthermore, this crop is more sustainable in Central Italy than flue-cured tobacco due to a roughly fivefold reduction in water input. Since honeybees (*Apis mellifera*) frequently visit an actively flowering tobacco field, this crop could even contribute to mitigate the decline of these important pollinator populations. However, either replacing flue-cured tobacco cultivation or being a novel biofuel resource requires an increasement of "Solaris" seed yield. Alternative strategies can be adopted such as "Solaris" cultivation in agriculture areas where a favorable growing season can lead to multiple seed harvests. Moreover, the crop profitability can be increased by collecting, after seed harvest, the remaining green tissues (stems and leaves) for biomass production. This biomass could be put inside an anaerobic digester to obtain biogas. Further studies are necessary to make the best use of tobacco as a novel industrial crop.

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