OPTIMA Report Summary

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Final Report Summary - OPTIMA (Optimization of Perennial Grasses for Biomass Production)

Executive Summary:

1. Executive Summary

OPTIMA project (OPTimisation of perennial grasses for biomass production in Mediterranean Area, Grant Agreement 289642) was a 48-month FP7 funded project under the theme: KBBE.2011.3.1-02 Perennial grasses: optimising biomass production - SICA. In total 21 institutions belonged to OPTIMA, 6 of which were small medium enterprises. Furthermore, 3 SICA members, namely Argentina, China and India enriched the consortium. OPTIMA was coordinated by the University of Catania, Italy.

The OPTIMA concept arose due to big concerns for farming systems in the Mediterranean Area. The Mediterranean climate is in fact characterized by hot and dry summers, and most of the global models on climate show that the water supply will be much lower and the air temperatures significantly higher in short term, especially during the summertime. This poses serious threats for several conventional crops, particularly in marginal areas. Beside that, there is a growing general awareness and consensus among politicians and farmers on the need to reduce the environmental loads and the energy inputs of farming systems through the introduction of novel species, optimization of agronomic inputs and natural resources, primarily water.

Therefore, alternative crops with high tolerance to hot temperatures, to limited water supplies and to other constrained factors have to be urgently identified, adapted or developed.

In general, perennial grasses are drought resistant crops and recently have attracted steady interest due to their extensive environmental benefits both at global and agricultural community-scale. Compared to traditional row crops, perennial grasses generally require lower energy inputs (fertilizers, pesticides etc.), can be grown on less productive cropland and provide benefits in terms of soil structure and stability (e.g. reduced soil loss, erosion and runoff), soil quality (e.g. increase in soil fertility, organic matter and nutrient retention) and biodiversity (e.g. cover for native wildlife).

The main objective of the OPTIMA project was to identify high-yielding perennial grasses suitable for the Mediterranean area, optimizing the production chains in order to provide stable source of biomass raw material and new plant derived bio-products. Furthermore, OPTIMA explored the potentialities of selected perennial grasses on less productive marginal lands. An interdisciplinary approach involving physiology, biotechnology, agronomy, socio-economic and environmental analysis at different scale levels was undertaken with the aim at tackling specific bottlenecks of perennial grasses in the Mediterranean area and to create alternative end-use chains.

Project Context and Objectives:

2. Project context and objectives

Recently, the twenty-first session of the Conference of the Parties (COP21) serving as the meeting of the Parties to the Kyoto Protocol (CMP) took place from 30 November to 11 December 2015, in Paris, France. It was recognized that “climate change represents an urgent and potentially irreversible threat to human societies and the planet”. The Parties stressed on the “urgency of accelerating the implementation of the Convention and its Kyoto Protocol to limit the temperature increase to 1.5 °C above pre-industrial levels, to promote universal access to sustainable energy in developing countries through the
enhanced deployment of renewable energy” (UNFCCC, 2015).

Concerns are unequivocal, therefore measures to mitigate global warming and to the fossil fuel dependences have to be urgently adopted. Great effort has been placed on the exploitation of crop residues, forest byproducts and other forms of lignocellulosic plant biomass as feedstock for bioenergy/biofuel production. Lignocellulosic bioenergy crops are expected to play an important role in achieving long-term goals for energy policy in cutting CO2 emissions and contribute to the maintenance of energy supplies. Among lignocellulosic species, perennial grasses are attractive feedstocks to produce advanced biofuels or bio-based products because of their non-edible nature, high biomass yields and cellulose/hemicellulose composition. As high yielding species, perennial grasses could lead to more energy production than annual or woody species. They are also recognized for providing substantial environmental benefits in terms of greenhouse gas savings, soil erosion mitigation, soil fertility, and increased biodiversity.

From the beginning of the 1990s, several European projects (Miscanthus productivity networks, Giant reed network, Switchgrass for Energy, Bioenergy Chains, 4FCROPS, EUROBIOREF, etc.) addressed miscanthus (Miscanthus spp.), switchgrass (Panicum virgatum L.), and giant reed (Arundo donax L.) for energy or biorefinery purposes. The present OPTIMA project was inspired by the aforementioned projects with the aim to provide insights into perennial grasses grown on marginal lands in the Mediterranean area.

Indeed, serious concerns have been expressed over the competition for land use between food and non-food cropping systems. Currently there has been a long debate going on the use of land for food or fuel and it has repeatedly suggested that non-food crops should be cultivated on marginal lands in order to avoid land-use competition with food and its adverse effects on food security.

However, there is no clear definition of “marginal land” or, to be more precise, there are too many. A vague definition of marginal land does not help the discussion and cannot provide a solid basis for agricultural policy. Besides, the dilemma “food or fuel” is at the moment answered, at least in Europe, in favour of the first, with the general attitude against any drastic changes in land use. Indeed, to ensure that bioenergy crops contribute to GHG emission savings and that their overall sustainability is maintained, a regulation in order to avoid undesirable land use change (LUC), caused by the expansion of bioenergy feedstock production, is required. This further restricts energy crop cultivation on land with lower economic rent and productivity, where agriculture is financially less attractive.

Although in most cases this is not the real problem, it seems that a more clear-cut expression, such as “unused lands”, may facilitate the dialogue, because it better captures the meaning of the issue being discussed.

The word marginal has been extensively used, for example in economics, to define any quantity at or just beyond the “border” or “margin”. For example, marginal cost and marginal revenue are common expressions, indicating the incremental cost or revenue generated from the production or sale of one more unit of output. Marginal utility is the increase in utility gained from consuming one more unit of some product. Since the value of such magnitudes depends upon the current or pre-existing level of production, consumption, sales, etc., the “margin” is specified in relation to the currently observed situation.

In agriculture, the use of the term marginal land (or surplus land, fallow land, set-aside land, pasture land, abandoned land, non-food land, low rent land) denotes land which is less attractive for cultivation or less productive and is often contrasted with favoured or prime or arable agricultural land.

In a general sense, marginal land is assumed to be a land of low quality for modern intensive cropping systems that is not being used for the current production needs of conventional crops. However, little research has been done to determine exactly what defines land as marginal, the quantity of this land, its physical, chemical and biological properties, and the opportunity cost of these so-called “marginal” areas (Dauber et al., 2012).

This ambiguity caused vivid discussions in the course of the 4-year project since no clear definition of marginal land is still available. In OPTIMA the concept of marginal land has been related to those lands presenting biophysical limitations in Mediterranean area (e.g. soil salinization, water limitation, steep slope and contaminated soils).

Salinization is considered by the United Nations Environmental Programme (UNEP) to be the second largest cause of land loss. According to the Food and Agriculture Organization (FAO) of the United Nations, it is estimated that 34 million hectares (11 % of the irrigated area) are affected by some level of salinization. Therefore, salinity is one of the most important challenges facing the supply of food to the world’s population in the future (Wilhelm, 2014). On the other hand, increases in the intensity and/or duration of water stress may occur across the globe due to recent observed changes in climate, especially in the
Mediterranean region and West Africa. According to the FAO, in 2011, 38.47% of the world’s total land area was dedicated to agriculture and only 6.48% of this agricultural area was equipped for irrigation. Moreover, it is very likely that arid and semi-arid regions, where evapotranspiration dominates, are becoming more saline (IPCC, 2013). Both salinity and water stress are among the most important environmental limitation affecting plant growth, development and crop yield in Mediterranean environments.

Soil erosion is one of the biggest environmental threats in the Mediterranean area as it causes pollution of water bodies, critical losses of water and nutrients, soil organic matter, and soil biota from the natural ecosystems (SOER, 2010). The long dry period followed by heavy bursts of erosive rains, falling on steep slopes with fragile soils, results in considerable amounts of soil erosion that, especially through the loss of organic matter and nutrients, leads to a reduction of cultivable soil layers and soil fertility (Van Rompaey et al., 2005). Overall, this causes a loss of crop productivity that could be initially replaced with an increase of fertilizers, but in the end leads to land abandonment (Pimentel et al., 1995; Crosson, 1997; Cerdan et al., 2010).

Unlike many compounds used in industry, medicine, and agriculture, heavy metals and metalloids cannot be degraded and are an integral part of the natural biogeochemical cycles (Benjamin and Honeyman, 1992). Heavy industries such as the metallurgical and petrochemical, municipal wastewaters, fertilizers and pesticides, emissions from waste incinerators and cars, mining and construction are some of the anthropogenic activities that are increasing the dissemination and mobilization of heavy metals (Alloway, 1995; Kabata-Pendias, 2011). These processes significantly contribute for the degradation, contamination, and pollution of the ecosystems, inducing a serious threat to the environment and public health. In particular, they might cause marginality of soils through the degradation of their quality—inducing the reduction of crop yields and the quality of agricultural products, desertification, and the loss of ecosystem services (Fergusson, 1991; Garbisu and Alkorta, 2003; He et al., 2005; Dauber et al., 2012).

Due to morphological and physiological traits, perennial grasses have the potential to survive over prolonged dry periods, to act as carbon sinks and filter systems for removing agrochemicals from water before these pollutants reach groundwater bodies. Moreover, the use of perennial grasses for phytoremediation with waste water and sludge’s have the potential to improve their economic and environmental viability. Perennial rhizomatous grasses in particular provide benefits in terms of soil structure and stability preventing the risk of soil erosion.

The core species investigated in OPTIMA are the rhizomatous Miscanthus spp. Arundo donax and Panicum virgatum. Furthermore, other perennial grasses less investigated and widespread in Mediterranean area were tested as potential bioenergy crops. Cardoon (Cynara cardunculus), belonging to the Asteraceae family, was also considered in OPTIMA since perennial and native of the Mediterranean area.

In order to promote the development of perennial grasses grown on less productive marginal lands that in the long term will foster the sustainable development of the EU biobased economy and will contribute to achieving energy and climate targets, the following specific objectives were pursued:

- To study plant and leaf physiology of switchgrass, miscanthus and giant reed with respect of early emergence, osmotic regulation, photosynthesis and transpiration under salinity and water stress conditions, regulation of metabolite degradation during senescence, improvement of biomass characteristics to increase capacity for rapid drying [WP1].

- To allow the generation of novel varieties of plants through SNP marker development using new developed technique, genotyping promising clones, phylogeographic analysis and markers utilization [WP2].

- To collect and characterize both the endemic perennial grasses and novel varieties of plants suitable for South Europe that will address the challenge of climate change and optimization of agricultural practices for switchgrass, miscanthus and giant reed [WP3].

- To determine the farm-scale productivity for the selected perennial grasses for yields maximization in marginal lands and to tackle specific bottlenecks (cultivation techniques such as intercropping in marginal lands, productivity in marginal large fields and long term productivity of existing large scale plantation, harvesting and storage strategies and logistic to delivery to market) [WP4].

- To investigate different pathways for plant derived bio-products, bioenergy products (conversion paths & technical specifications), biomass requirements for different conversion technologies (potentials, technical specifications and logistics), market analysis and regulatory framework for bioenergy and bio-based products in Southern Europe [WP5].

- To exploit specific environmental implications of perennial grasses on marginal lands, with particular emphasis on the
assessment of CO2 and water exchange between canopy and atmosphere, root development and carbon storing in the soil, energy balance, phytoremediation, soil erosion mitigation and environmental impact assessment [WP6].
- To provide an integrated assessment of sustainability of perennial grasses based on life cycle assessment (LCA), cost, socio-economic implications, energy balance and SWOT analysis from the agricultural production to the industrial supply [WP7].
- To disseminate project activities and results among stakeholders [WP8].
- To coordinate and manage the OPTIMA project [WP9].

Project Results:
3. Main Scientific and Technical results
3.1 Overall concept

OPTIMA integrated a biology system approach for perennial grasses in the Mediterranean environment, addressing the suitability of these species for their introduction in marginal lands. To achieve the goals of the project the work has been structured in nine Work Packages.
- WP1 Plant and leaf physiology;
- WP2 Plant biotechnology;
- WP3 Plant agronomy;
- WP4 Farm-scale productivity of perennial grasses on marginal lands;
- WP5 Energy products and plant derived biomaterials;
- WP6 Environmental studies on perennial crops;
- WP7 Integrated assessment of sustainability;
- WP8 Dissemination;
- WP9 Project management.

Seven out of nine WPs were of RTD nature, one WP addressed findings dissemination (OTH) and one WP committed to the project management (MGN). OPTIMA approach has been to link the research proposed here by including industrial end-users in the project (IFEU, SPAPPERI, INDEAR, BTG, PRIMUS, 2ZK). This should allow the output of this research to develop in a commercial context as rapidly as possible. The major goals of this multidisciplinary network were to evaluate the existing genotypes under stress conditions; to characterize and deliver novel species; to deliver sustainable crop management practices; to evaluate the industrial production of bioenergy and added value bio-products; to assess the environmental impact through an integrated assessment of sustainability criteria, to disseminate the achieved findings at different level (local, regional, national, international) and to manage the project.

Overall, OPTIMA aimed at increasing the awareness on perennial grasses for energy and plant derived bio-products and visibility of the scientific achievements generated in this project to plant breeders, farmers, scientists, policy makers and consumers.

3.2 WP1 Plant and leaf physiology
3.2.1 Aim
The objective of the WP1 was the study of physiology at plant and leaf level of switchgrass, miscanthus and giant reed with respect of early emergence, osmotic regulation, photosynthesis and transpiration efficiency under salinity and water stress conditions, regulation of metabolite degradation during senescence to improve biomass characteristics and to increase capacity for rapid drying.

This WP was under the coordination of partner UB; contribution was given also by UCD, UIB, INDEAR, IBERS and PRIMUS. WP1 was split into two main tasks where C4 and C3 photosynthetic pathways crops were investigated separately. Task 1.1 Physiology of switchgrass and miscanthus (C4) and Task 1.2 Physiology of giant reed (C3).

3.2.2 Project Scientific and Technical Results
Task 1.1 Physiology of C4
Early emergence studies
Test on sprouting of stem-buds was done at PRIMUS in order to prove the sprouting potential of lateral buds of Miscanthus
derived from different origin and time by removing the top part of the stems.
The time gap (winter) between the growing season of Miscanthus stems and its possible utilization was solved by stem-storage. Pre-treated and non-treated stems were overwintered in pit and the bud-viability was examined. The water-pre-treated stems survived better than un-treated stems. Buds were still alive in the case of the pretreated Miscanthus stems after 11 months pit-storage contrary to totally dead buds on non-pretreated stems.

In order to improve the sprouting activity of Miscanthus a stem-buds infiltrating process and instrument prototype was developed. After activation the most of buds developed contrary to non-infiltrated samples. To test the activating instrument and experiments, the different stem cuttings were placed into basal activating solution with low pressure (approx.: 0,001 atm.) and stored in it 10 days long as control. The fast activating-process took only 1 hour. The shooting rate of stem buds after 1-hour-long intensive activation process was 2,5-3 fold higher than the shooting of stems after 10-day-long soft inducing. Similar results were found with overwintered stems.

The shooting efficiency (speed and rate also) of Miscanthus stem-buds were significantly better in higher temperature than in low temperature.

Effect of different types of phytohormones and agents were studied with different concentrations. New shoots and roots were examined. Some auxins caused a weak inhibiting effect especially in higher concentrations. Solutions of 2,4-D, NAA and 2,4,5-T caused strong inhibition in higher concentration on shooting. IAA in lower concentration caused improvement on shoot development, but had inhibition in high concentration. The adventitious aerial root development of original stems was generally improved by auxins. The cytokinins and cytokinin-like agents showed positive effects on shooting but the adenine-solution inhibits the growth in high concentration.

In switchgrass, UCD carried out germination test at 6 ºC during 10 days on 13 switchgrass cultivars (Upland: Blackwell, Trailblazer, Shelter, Cartaghe, Shawnee, Cave-in-rock, Sunburst and EG2101; Lowland: Kanlow, Cimarron, Alamo, EG11101 and EG11102). CO2 assimilation, chlorophyll fluorescence, biomass yield and recovery were assessed.

All ecotypes showed reductions in photosynthesis after low temperature exposure. It was observed a variable recovery after exposure. Uplands cultivars are less sensitive to low temperature exposure (better average recovery rate) than lowland ones. Fresh biomass data suggests compensation through increases in leaf area per plant. It was shown a limitation due to reductions in the catalytic activity of RUBISCO and/or the supply of carbon dioxide to the bundle sheath cells.

Miscanthus response under salinity and water stress

A diversity trial of 244 Miscanthus genotypes in 4 replicate blocks was analysed at IBERS in order to identify traits associated with early emergence. The trial contains genotypes from M. sinensis and M. sacchariflorus species groups and also hybrid genotypes such as the commercial genotype M. x giganteus. Plants were screened for chlorophyll and scored for visual signs of leaf stress. Selected genotypes that spanned the range of responses were analysed using chlorophyll fluorescence to confirm that selections encompassed a range of stress responses. Finally, selected genotypes were sampled for metabolomics analysis which included confirmatory chlorophyll assessments.

Several Miscanthus genotypes had been identified in our OPTIMA project previously from a trial of Miscanthus growing in Catania, Italy (UNICT). These genotypes outperformed M x giganteus (C2) for yield under the drought conditions. These genotypes were selected for further analysis including examples of drought tolerant and sensitive types which had been confirmed from previous experiments in years 1 & 2. Detailed physiological experiments were completed in year 3 and the data from this experiment was analysed in year 4 and forms the basis of the report for deliverable 1.3 (delivered at M48). The highlights from this experiment are discussed below. The main objective of the research in OPTIMA was to maintain biomass accumulation under water limited conditions. In respect to the above ground DM, seven out of nine genotypes did not show any response to the treatment effect, except OA8 (p<0.05) and OA11 (p<0.01). This may be because the treatment was not sufficiently severe to elicit and response in all genotypes however, height is one of the most sensitive indicators of stress in Miscanthus (Ings et al., 2013). At 20% F.C. (field water holding capacity) most of the genotypes did not show significant differences between control and stressed plants. The control genotype (C2) showed a significant reduction in final height (p<0.05) under drought. Final height was differentiated mainly due to strong genotypic effect (p<0.001) and showed a treatment effect at p<0.1 indicating that the treatment was sufficient to elicit a response in C2 (M. x giganteus) and that the novel genotypes were more tolerant of stress in terms of biomass accumulation and elongation growth.

To understand why those genotypes are not changing their biomass under drought we tried to answer: 1) how each parameter
is affected, 2) what parameters are affected by 20% F.C 3) how these parameters are changing in each genotype, and 4) if there is a species pattern. Genotypes showed different response of biomass partitioning between above and below ground dry biomass. The genotypes OA11, OA8, OA7 and OA10 were significantly higher for the ratio of Below/Above dry biomass investing more in the belowground dry biomass, in comparison, genotype OA1 invested more biomass aboveground (p<0.001).

Maintaining the biomass under water limited conditions is a major indicator of tolerance. Above ground dry biomass was significantly reduced under treatment conditions (20% F.C.) for the genotypes OA8 and OA11. Genotypes OA8 and OA11 share common response trends under drought such as investing more in the roots and senescing and decreasing their evapotranspiration towards the end of the experiment; however, they also have some opposing response trends, possibly because they belong to different species. Those responses include the final height, OA8 the shortest genotype, but OA11 was the tallest one (when compared to the other genotypes), OA8 had a significant less leaves under drought whereas (when compared to control plants), yet, OA11 had the maximum number of leaves over all genotypes. Also OA11 had reduced vitality in respect to Performance Index (PI) calculated from chlorophyll fluorescence assessments. The genotype OA8 had the lowest assimilation rates that did not differ from each other and were reduced towards the end of the experiment, while OA11 had the highest among all genotypes. Finally, OA8 had the lowest capacity of photosynthesis (ETRmax) and light intensity in which photosynthesis is saturated (Ik) over all genotypes.

The genotypes of which the biomass was not affected under drought changed less physiological parameters and did not show any changes in the chlorophyll content (except C2), the assimilation rates and evapotranspiration that remained constant throughout the experimental period. Possible trends showed the M. sinensis OA5 and OA7 originated from Japan, that had the lowest intrinsic Water Use Efficiency, yet OA5 reduced the Fv/Fm and the PI, while OA7 had the highest efficiency in harvesting light (α). M. sinensis OA2 and OA4 originated from China, had also similar responses, and OA4 also showed highest efficiency in harvesting light (α).

A second salinity experiment was completed in year 3 and data to determine the quantitative responses to salinity stress in M. x giganteus analysed and written for publication in year 4. Many marginal areas that will be suitable for growing biomass crops exhibit a combination of both low moisture availability and salinity stress. Therefore to test if drought tolerant genotypes also tolerated combined drought and salinity tolerance in year 4 a combined salinity and drought experiment was completed using 3 selected genotypes and M. x giganteus.

The response to salinity is not well defined in Miscanthus so initially the experiments focussed on determining the impact of saline conditions on growth, physiology and biochemistry of M. x giganteus. M. x giganteus was cloned and after 3 weeks growth the plants were treated with 9 different concentrations of NaCl (0, 30, 60, 90, 120, 150, 180, 210 and 240 mM), (n=5). Biomass production was significantly reduced in concentrations over 30 mM with the maximum decrease in above ground biomass occurring at 150-240 mM. The below ground biomass decreased significantly at 210 and 240 mM of NaCl. Below ground partitioning (root/rhizome, fresh weight) was increased significantly in NaCl concentrations over 0 and 30 mM. WUE was decreased by 40.6% in concentrations over 90 mM NaCl. The response of stomatal conductance over time and final height were also reduced by 40.3% and 22% respectively in response to rising NaCl concentrations. Salinity induced significant changes in the estimate of the PSII maximum efficiency within light-adapted material (Fv’/Fm’) while no significant differences were observed in Fv/Fm. The non-significant differences in quantum yield of dark adapted leaves (Fv/Fm) suggests stable photochemical conversion efficiency of PSII is continuing with salinity stress. No differences were observed in the photochemical and non-photochemical quenching coefficient (qP, NPQ), Yield and the physiological state of the photosynthetic apparatus (Fo/Fm). Proline accumulation increased as the lipid peroxidation reached the highest level at concentrations over 120 mM.

During the time period of the experiment there were significant reductions in key physiological parameters and a reduction in biomass and properties associated with combustion quality. The overall performance of M. x giganteus was affected at NaCl concentrations above 60 and 90 mM NaCl. This level of salinity, which equates to 5.4 and 8 dS m-1, proved to be the tipping point where the effect of increasing salinity significantly reduced plant biomass and increased ash content. The reasons for this reduction in biomass can be attributed to reduced photosynthetic efficiency, and reduced transpiration. These are normal plant responses that are commonly associated with low water availability. Whereas water availability was not a limiting factor in this experiment, the osmotic stress caused by the NaCl dissolved in the water caused an inter-leaf osmotic stress which
triggered responses. It should be emphasized that in this study, 90 mM NaCl proved to have a significantly detrimental effect on M. x giganteus and that this was without water limitation. If the negative stresses of water limitation and salinity were combined, then there will be an increased reduction in plant performance, which will most likely occur at a lower NaCl concentration.

In the present study, M. x giganteus biomass production was reduced only at moderate to high salinity concentrations, while the physiology was mainly affected at higher concentrations and was dependant on the stress duration; it also demonstrated enhanced accumulation of osmoprotectants and low lipid peroxidation. The experimental data was used to determine an equation that could be used to predict the reduction from the maximum yield on non-saline soils so that the actual yields could be predicted on saline soils. Based on the map of potential M. x giganteus yield only a small area in EU27 would pose restrictive limitations in biomass production of Miscanthus due to salinity, 9,601km2 is expected to result in M. x giganteus dry matter losses higher than 48% at saline soils between 3-15 dS m-1 NaCl. Miscanthus could therefore be a potential candidate to be tested for phytoremediation and biomass production in areas with low to moderate salinity.

Switchgrass response under salinity and water stress

Three Switchgrass varieties - Alamo, Kanlow and Trailblazer - were sown in a mixture of John Innes compost No.2 perlite and vermiculite (2:1:1, v:v:v) in 4.5L pots (one plant per pot and 60 pots per variety) at UCD. Plants were grown in a greenhouse at 25/15 ºC day/night under natural daylight. Plants were irrigated manually as needed during the experiment (electrical conductivity of water: 1 mS cm⁻¹). Salt stress was induced by NaCl addition (50 and 100 mM in the irrigation water) two months after sowing. Each pot was irrigated with 500 cm³ of the appropriate salt solution twice a week, whereas the control (0 mM NaCl) treatment was irrigated from the surface with non-saline water.

Harvests were performed at month 7 and 9 to assess the effects of delayed harvesting on biomass production. At each harvest, total dry matter (DM – g/plant) of the different plant parts were determined (n=6). Delaying the harvest had different effects on the studied genotypes. Alamo and Trailblazer did not show any changes in biomass yield between harvests. Whilst Kanlow, however, showed a decrease in yield from one harvest to another for the control and the 100 mM treatment, probably due to leaf loss over that period.

In general, lowland genotypes (Alamo and Kanlow) showed a bigger reduction in biomass across the different salinity levels, whereas Trailblazer did not show a salinity-dependent reduction in final yields. All the genotypes showed a reduction in Relative Water Content (RWC) except for Alamo in the control treatment and Kanlow at the highest salinity level, indicating that a delayed harvest could help reduce the water content and therefore improve quality of biomass if there are no biomass losses.

Salt and water stress tolerance of several switchgrass cultivars performed at INDEAR contributed to the interpretation of tolerance response of switchgrass cultivars. Laboratory and greenhouse experiments showed that cultivar Experimental I and II exhibited a greater tolerance response to recreated conditions of salin and water stress when compared to other well-known switchgrass cultivars. On-going experiments in the field will certainly confirm or complete these previous evidences.

Task 1.2 Physiology of C3

Early emergence studies

The general objective of the experiments carried out by UIB was to study the effect of mycorrhizal inoculation in giant reed (Arundo donax L.), paying special attention to plant performance under marginal lands conditions (water stress, salinity and nutrient deficit) and to plant propagation. It was evaluated the gas exchange, biomass production and silicon content in 1-year potted plants under growth chamber conditions. Plants were grown at 3 salinity levels and 2 levels of [P]. Mycorrhizal infection increased plant biomass production about 41% under non-saline conditions. This increase in biomass was maintained under moderate salt stress with inoculated plants showing 14% higher values than non-inoculated ones. However, severe salinity stress eliminated this positive effect of mycorrhizal inoculation on biomass production. Mycorrhizal appeared to decrease the shoot-to-root Si translocation in Arundo donax plantlets.

Eight arbuscular mycorrhiza species (AMF) were found at the A. donax rhizosphere: Claroideoglomus etunicatus, Clareidoglomus claroideum, Diversispora sp., Funneliformis mosseae, Glomus badium, Rizophagus intraradices, Glomus irregularis, Septoglomus constrictus. Two of the species found in the rhizosphere of A. donax are two of the most common species in commercial arbuscular mycorrhiza inoculum: F mosseae and R. intraradices. Both species have been tested demonstrating their ability to infect Arundo donax facilitating its growth.
Early inoculation with AMF of micropropagated plants of A. donax can be an effective method to improve the acclimatization process and plantlet quality. Moreover, the study carried out during the last year of the project opens new lines of research to investigate the long-term effect of inoculation on A. donax aerial biomass production, the role of AMF on plantlet herbicide resistance and the search for more effective AMF inoculants. Mycorrhizal technology can be a good strategy for improving the plantlet production process of this promising energy crop and merits further exploration.

Arundo donax under salinity and water stress

Different Arundo donax ecotypes were grown in plastic pots containing 5 L of peat: vermiculite (3:1:1) and were irrigated with a complete Hoagland solution (Hoagland and Arnon, 1950).

Five of the ecotypes used (namely Fondachello, Tortorici, Cefalú, Agrigento and Licata) were collected from the Experimental Fields at UNICT, while two ecotypes (namely Martinensis and Granadensis) were collected in different riparian areas of Spain and a commercial ecotype “Piccoplant” was provided by a private company (Piccoplant, Oldenburg, Germany).

The average temperature and vapour pressure deficit (VPD) in the greenhouse during growth was 25/15ºC day/night and 0.75 kPa, respectively. Relative humidity ranged from 40 to 65% and the maximum PPFD was ~1000 µmol m-² s-¹. Three months after planting, plants were separated into four treatments. Half the plants were subjected to water stress by withholding water until 25% of field capacity (FC). Then, well-watered (maintained at FC) and water stress plants were equally divided between salinity (e.g. 16 mS cm⁻¹ Hoagland solution) and non-salinity (e.g. 1 mS cm⁻¹ Hoagland solution). Saline solution was prepared by adding NaCl (PANREAC, 99% Sodium Chloride) to a complete Hoagland solution until the appropriate saline concentration was reached. Consequently, a total of three plants per specie were subjected to the following treatments: (i) well-watered with non-saline solution (WW S⁻), (ii) water stress with non-saline solution (WS S⁻), (iii) well-watered with saline solution (WW S⁺) and, iv) water stress with saline solution (WS S⁺). Plants were subjected to these treatments for two months and the plants were measured every fifteen days (i.e. T15, T30, and T45) until the end of the experiment (i.e. T60).

By measuring several physiological, morphological and biomass traits, it was found that giant reed might be more tolerant to salinity than to water stress. Salinity and water stress mainly affect stomatal conductance (gs), generating stomatal closure in order to prevent dehydration of the plant, and consequently decreasing photosynthesis. There was a differential response to salinity and water stress among the eight ecotypes of giant reed, with the most water stress-resistant being Agrigento and the most salinity-resistant being Martinensis and Cefalú.

Fondachello, Cefalú and Licata were the most salinity resistant in relation to increasing salinity levels. In order to benefit from a constant performance and to obtain a maximised yield of biomass, which is the main objective of growing perennial grasses, we propose Martinensis and Piccoplant as the most suitable ecotypes for growing in both stress conditions for biomass production.

However, it is important to note that this screening against salinity and water stress was performed under greenhouse conditions in pots, where the growth and behaviour of plants might be different than in field conditions. On the other hand, we have imposed a high level of stress, which will be rare under field conditions, but this approach was used to physiologically characterise the behaviour of giant reed under adverse conditions.

Senescence and regulation of metabolite during stress of C3 and C4

A. donax ecotype “Piccoplant” and P. virgatum cv “Alamo were grown during six months in a greenhouse at the Experimental Field Service of UB in plastic pots of 5 L of peat: perlite: vermiculite (3:1:1). In order to study the response to different water stress levels, six months after planting, plants were subjected to three water treatments during a during 21 days: (i) Control (C, 100% Field Capacity), (ii) Mild Stress (MS, 50% FC) and (iii) Severe Stress (SS, 25% FC). 13C and 15N labelling, δ¹³C and δ¹⁵N of total organic matter (δ¹³C TOM and δ¹⁵N_TOM), δ¹³C determination of total soluble carbohydrates sugars (δ¹³C TSS) and δ¹³C of dark-respired CO2 determination (δ¹³C R) procedures were used.

It was observed a decreases in photosynthetic and biomass parameters in both species between treatments due to water stress. Differences in metabolic pathways between C3 and C4, as photosynthesis, sugar metabolism and fluxes during the reductive pentose phosphate pathway could be the causes of the isotopic differences in control conditions. Increases in δ¹³CR and δ¹³C_TOM (or a decrease in photosynthetic discrimination) due to WS are expected as a consequence of decreases in gs and therefore in the intercellular partial pressure of CO2 (ci). In the results of the isotopic composition in (200 µl) soluble sugars and in (200 µg) starch of giant reed and switchgrass, we observed the δ¹³C increase in T1 and progressively decrease in T2. Between four organs studied (root, rhizomes, stems and leaves), the stem and rhizome showed higher δ¹³C
concentrations above the root and leaf. This suggests that stem and rhizome were permanent sink organs.

3.2.3 Impact on other Work Packages
Direct stem transplanting of Miscanthus after stem-bud activation was successfully achieved also in field conditions (in connection with WP3). The time of stem transplant, influenced shoot density and, hence, biomass yield. An effect of air temperature was observed, with higher minimum temperatures enhancing new shoot emission, suggesting that temperatures close to 20 °C would be ideal for the successful establishment of activated stem cuttings. An important factor to take into consideration is, however, the availability of water.

The overall performance of M. × giganteus was affected at NaCl concentrations above 60 and 90 mM NaCl. This level of salinity, which equates to 5.4 and 8 dS m⁻¹, proved to be the tipping point where the effect of increasing salinity significantly reduced plant biomass and increased ash content, properties associated with combustion quality (WP5).

Delayed harvest could help reduce the water content (such as relative water content) and therefore improve quality of biomass if there are no biomass losses and mechanization is not affected (WP5).

In giant reed, early inoculation with AMF of micropropagated plants can be an effective method to improve the acclimatization process and plantlet quality (WP3&WP4).

3.2.4 WP1 Deliverables
Overall WP1 results are available on the deliverables (D1.1 to D1.12) submitted throughout the 4-year project.

3.3 WP2 Plant biotechnology

3.3.1 Aim
The objective of the WP2 will be the molecular and genomic study of the promising grasses (selected from WP1) and development of large-scale molecular markers based on it, in order to provide important information and efficient molecular tools for collecting, characterizing and utilizing these grasses.

Since few genomic information and SSR/AFLP are low throughput the next generation sequencing techniques was used. Next Generation Sequencing (NGS) technologies have already been used for a variety of applications, such as developing SNP-based markers in a number of plant species both where a reference genome is available and where it is not. Using the NGS technology, a large-scale of genomewide distributed SNP markers could be developed. The genetic variation of the collection can be assayed using the genotyped SNPs. Genetic diversity, allelic frequency, taxonomy, and phylogenetic analyses can be performed based on the genotypic data. Core collection can be effectively constructed using the genotypic data. Genetic mapping and marker-assisted selection will facilitate improvement of the grasses.

This WP was under the coordination of partner INDEAR; contribution was also given by HZAU, and UB. WP2 was split into the following tasks: Task 2.1 Next Generation Sequencing for SNPs development of switchgrass and Task 2.2 Biotechnology of miscanthus and giant reed.

3.3.2 Project Scientific and Technical Results
Task 2.1 Next Generation Sequencing for SNPs development of switchgrass
The main goal was to develop Switchgrass SNPs for the genotyping platform using NGS technologies. Eight different ecotypes were used (from tetra- to octoploids). Two experiments were performed at INDEAR for the 454/Roche platform: RNA sequencing and Genome Reduction (GR) sequencing. Results were not as expected for GR due to problems during wet lab stage of the protocol, while RNA-seq results were good but didn’t have enough coverage to support all SNVs. To complement the poor obtained results, additional NGS sequencing was performed. Deep transcriptome sequencing was performed using a full IlluminaHiSeq 1500 rapid run, with 2x150 bp paired-end reads, obtaining 95.5 Gbp of data (632.4 Mi reads). Analysis was performed using GATK RNA-seq Variant Discovery pipeline. Almost 102k high-quality SNVs (~96k SNPs) were obtained using this method. RNA-seq showed to be a suitable platform to develop HQ SNVs for species where genome/transcriptome reference is available. Also, deep transcriptome sequencing enabled new projects opportunities with the already sequenced data, like improve P. virgatum transcriptome/gene prediction, identify novel transcripts/genes, study differential gene expression between ecotypes or analyze expressed pathways.

Greenhouse evaluation of the selected salt tolerant candidates was done. Though crosses have been partially delayed due to low germination power of original seed lots this problem has been partially solved by developing an in vitro plant propagation protocol for the different switchgrass cvs parental materials. Plants have been grown under controlled conditions in
greenhouse with supplemented light so as to favor plant growth. From the information generated from the Genomics and Bioinformatics Platform we have selected a marker (Marker 1) that presents a polymorphism level detectable by High Resolution Melting (HRM) between switchgrass Experimental 1 (EI) and Experimental 2/Summer (EII-S) parent plants.

In conclusion, three different strategies were used at INDEAR so as to develop high quality SNP markers: Genome Reduction 454-Roche + Transcriptomics with 454-Roche and RNA-seq with Hiseq Illumina.

RNA-seq shows to be a suitable platform to develop high quality SNP markers (when coverage is enough) for species where genome and/or transcriptome references are available. So as to complement the previous results an additional NGS experiment was performed. A complete Rapid Run sequencing was performed at an Illumina HiSeq 1500 sequencer at INDEAR. 102k HQ SNVs were obtained that uniquely identify an ecotype amongst the others. Almost 96,000 SNPs were obtained for the analyzed ecotypes. A solid base for future QTL detection has been established. The generated raw data and all the variants identified per ecotype totalize about 70 Gb. All these data will be split into partial files and will be made available to the Consortium partners.

Fast progress in DNA sequencing technology has made for a substantial increase in throughput and accuracy. Progress in genomics has been moving steadily forward due to a revolution in sequencing technology. For those reasons INDEAR has adopted the Illumina Hiseq 2000 Platform. Within OPTIMA we have been able to complement the pyrosequencing scientific approach by the high throughput transcriptome sequencing capacity of Illumina Hiseq 2000 platform. This platform not only offers a great accuracy but also has an enormous output data/run (about 600 Gb) while the 454 Titanium Roche generates about 0.5 Gb per run. This advantage has undoubtedly enriched the data volume and the general results.

After we complete the molecular characterization of all F1 candidate plants by the end of 2015, we will initiate the greenhouse evaluation of F1 candidates for salt tolerance. Field trials will be also completed by April-May 2016. All these results will also be available to the Consortium even if out the horizon of the project.

Task 2.2 Biotechnology of miscanthus and giant reed

Due to difficulties to send vegetable materials to China, UB was able to send only giant reed material to HZAU. Genotype-by-sequencing (GBS) technique was performed to get a genome reduction. 181 clones of Arundo donax have been genotyped. GBS is a suitable platform to develop high quality SNP markers, especially for the non-Ref genome species. As result, ~1.2 Million high quality GBS tags are obtained by the 181 clones with the sequencing depth about 288Mb each. The high density and quality SNP markers provide enough genetic diversity information. Combining different treatments done by UB in WP1, the associated loci with drought tolerance can be developed. To avoid influence of the genotype missing ratio, a strict filter set up. The missing ratio holds a significant decrease after filtering.

To build the genomic map of five kind of ecotype, the GBS was performed on 131 individuals. 367,017 SNP markers developed in total. Using those markers, a Neighbor-Joining tree was mapped to provide the information about the relationship between different ecotypes and internal ecotype. Lyophilized leaves under water stress (WS, 50 samples) or well-watered (WW, 50 samples) treatment were provided by UB (ecotype Piccoplant, Martinensis, Fondachello, Argentum, Granadensis) to build the genomic map. After receiving the Arundo donax leaves from UB, the DNA of 205 clones were extracted. The qualified DNAs were used for constructing the sequencing library for GBS. Then the GBS libraries were sequenced on the Illumina Hiseq 2000 platform. Sequencing was performed in single-ended mode with 100 bp read length. Finally we took advantage of the Universal Network-Enabled Analysis Kit (UNEAK) to call SNP markers based on the sequencing data. Based on the UNEAK, we achieved three result files, including the HapMap.hmp.txt HapMap.hmc.txt and HapMap.fas.txt. It records the SNP information, the tag counts of the SNPs in each clones and the sequence of the SNP tags, respectively. Then we counted the information in those files. Finally, 319,900 raw SNP marker numbers were developed from the GBS procedure. After filtering the tag counts depth <5 and >100, 52,842 high quality GBS SNP markers are reserved finally. Those high quality SNP markers will be used for genetic map construction to identify genes related to drought tolerance.

Using the developed 319,900 SNPs from the clones in drought tolerance experiment, a Neighbor-Joining tree was drawn to reveal their phylogeographic relationship. This result could provide the information about the relationship between each clone for analyzing sub-structure of this population. Using the 367,017 SNP markers developed from 131 individuals containing 5 ecotypes, such as Piccoplant, Martinensis,
Fondachello, Argentum, Granadensis, information about the relationship between different ecotypes and the internal ecotype for constructing genetic maps might be available.

In conclusion, GBS was a suitable platform to develop high quality SNP markers, especially for the non-Ref genome species. Finally, ~320K high quality GBS tags were obtained by the fifty clones with the sequencing depth about 288Mb each. Combing with the phenotypic data collected by UB, the genes related to drought tolerance will be identified in further. Furthermore, an AD-test can be performed through whole genome-wide to identify the associated loci with drought tolerance in further.

3.3.3 Impact on other Work Packages
A solid base for future QTL detection has been established. The generated raw data and all the variants identified per ecotype totalize about 70 Gb. All these data will be split into partial files and will be made available to the Consortium partners.

Fast progress in DNA sequencing technology has made for a substantial increase in throughput and accuracy. For this reasons INDEAR has adopted the Illumina Hiseq 2000 Platform. This advantage has undoubtedly enriched the data volume and the general results.

After completing the molecular characterization of all F1 candidate plants by the end of 2015, the greenhouse evaluation of F1 candidates for salt tolerance will be assessed. Field trials will be also completed by the April-May 2016. All these results will be available to the Consortium even though the project ended with important information on salt tolerance of switchgrass for marginal areas.

Combing with the phenotypic data collected by UB, the genes related to drought tolerance will be identified in further. Furthermore, an AD-test can be performed through whole genome-wide to identify the associated loci with drought tolerance. These results will be available to partners to identify ecotype suitable to drought conditions of marginal areas.

3.4 WP2 Deliverables
Overall WP2 results are available on the deliverables (D2.1-D2.6) submitted throughout the 4-year project.

3.4 WP3 Plant agronomy

3.4.1 Aim
The general objective of WP3 is to establish the most appropriate agronomic practices that lead to the optimization of the productivity of perennial grasses under Mediterranean climates. The specific objectives are: (i) to identify, collect, and test spontaneous native grass species with a great yielding potential under Mediterranean conditions; (ii) to enhance knowledge on the morphological and physiological traits and responses of native perennial grasses to environmental constraints in Mediterranean environments; (iii) to improve the agronomic practices such as plant propagation, crop establishment, development and harvest time; (iv) to evaluate the productivity of perennial grasses under salinity and drought stress conditions for their introduction in marginal lands.

This WP was under the coordination of partner UNICT; contribution was given also by CNR-ISPAAM, UIB, CRES, UNIBO, UPM and PRIMUS. WP3 has been split into two major tasks: Task 3.1 Characterization of endemic grasses and novel plant varieties adapted to southern European conditions; Task 3.2 Good agricultural practices for selected perennial grasses.

3.4.2 Project Scientific and Technical Results
Task 3.1 Characterization of endemic grasses and novel plant varieties adapted to southern European conditions
The list below shows the endemic species collected and tested by partners belonging to southern Mediterranean area in OPTIMA. The species Phalaris aquatica, Festuca arundinacea, Dactylis glomerata were used as cross-check.

- UIB: Dactylis glomerata, Ampelodesmos mauritanica, Piptatherum miliaceum (sin. Oryzopsis miliacea)
- CRES: Pennisetum purpureum (hybrid from China), Festuca arundinaceae, Phalaris aquatica (from Greece)

In Sicily Island (UNICT) all endemic grasses proved to be well adapted to semi-arid Mediterranean area, growing well in completely absence of agronomic input supply. Among the wild species tested, Saccharum spontaneum ssp. aegyptiacum, showed to be the best ideotype for southern environments of Europe in terms of biomass yield and water use efficiency. Net photosynthesis decreased as the water stress, temperatures and vapour pressure deficit (VPD) increased along the growing
season, however, the partial stomata closure allowed to loose little water via transpiration while CO2 was still up taken, improving the instantaneous water use efficiency. Ash content for thermochemical purposes is quite similar to other lignocellulosic, perennial grasses such as giant reed, miscanthus and switchgrass. NDF, ADF and ADL, representing the amount of hemicellulose, cellulose and lignin, respectively, useful for biochemical conversion were similar between the species and other monocot species and agricultural residues. Hemicellulose and lignin did not greatly change between autumn and winter harvest, while cellulose enhanced in winter as compared with autumn harvest. Moisture content decreased as the harvest was delayed.

In Greece (CRES) a comparison between the endemic and already known (switchgrass, miscanthus and giant reed) was attempted. Between the two groups (endemic vs known) of the perennial grasses it is clear that the known perennial grasses showed significantly higher yields compared to the first. Further to that, the mean yields of the endemic grasses at the end of the second growing period was 3.42 t/ha, while the corresponding yields for the known grasses was 19.62 t/ha (almost six times higher). Endemic and known perennial grasses were also compared in terms of calorific value, proximate and elemental analysis. The calorific value (both GCV and NCV) was higher for the known perennial grasses compared to endemic ones. The ash content was quite high in the endemic grasses, varying from 11.20 % (Phalaris aquatica – GR) to 13.05 % (Dactylis glomerata) probably due to protection mechanisms during hot summers. This aspect needs to be further studied. Miscanthus was the one that performed best in terms of ash (3.98%). It should be pointed out that the nitrogen content was quite low in the miscanthus biomass (0.19%). Elephant grass and giant reed had quite high nitrogen content on their leaves.

In Sardinia Island (CNR-ISPAM) the research allowed to identify three new species well adapted to the Mediterranean environment. Piptatherum miliaceum, in particular, showed a great DMY potential from the second year of cultivation, while Ampelodesmos mauritanica showed a slower growth and could be (well) evaluated for this trait in further years. Few data are currently available for Hyparrhenia hirta, whose plantation was carried out in 2014. Interesting, P. miliaceum is the only species that could be propagated by seed. This is one of the most important traits to be evaluated when comparing this perennial grass species with conventional bioenergy species (e.g. giant reed and Miscanthus x giganteus). All native species showed a good tolerance to summer drought. The harvesting of these species at the beginning of July (for P. miliaceum corresponds to the seed ripening stage, leaf are dried up; A. mauritanica is green), in addition, may be profitable for a further desiccation of biomass in the field, thanks to the high summer temperatures and dryness in Mediterranean environments. The photosynthetic activity in P. miliaceum plants in open field declines with the progress of the dry season and the leaf senescence. P. miliaceum tolerates drought following an avoidance mechanism, stopping the development of new leaves and tillers during the unfavorable season. The regrowth occurs when summer temperatures decrease, but it is strongly stimulated by autumn rains. In A. mauritanica, photosynthetic declines slower than in P. miliaceum with high temperatures and drought, as leaf remains green. In this species another mechanism, probably related to root system functioning or other physiological traits, acts during drought. Moreover, A. mauritanica re-sprout promptly after harvest in July, showing the ability to growth despite the absence of rains. Nonetheless, at the moment, we have no data to confirm this speculation and further research is needed to clarify the drought tolerance mechanism in A. mauritanica. The quality of biomass in P. miliaceum is comparable to those of perennial conventional species, for example switchgrass. Some negative traits are related to the high ash content of leaves and the higher contents of sulphur and chlorine compared to switchgrass. Nonetheless, the accessions differ partially for quality traits of biomass, and the existence of this variability can be advantageously used to set up a program of genotype selection aimed at obtaining plants suitable for the several bioenergy uses.

In the Balearic Islands (UIB) it was found that the use of non-conventional biomass species, like D. glomerata, P. miliaceum and A. mauritanica can be an alternative in Xeric Mediterranean areas where conventional biomass species show very low productivity due to large dry summers, that can last up to 5 months in those areas. Within P. miliaceum populations, data showed that there is a clear genetic difference between populations but also within populations, what suggests that this species presents a high genetic variation, what would be a basis for future breeding programs. The results showed that both, D. glomerata and O. miliacea, were successfully mycorrhizal inoculated (VAM). However, VAM inoculation had a greater effect on O. miliacea than on D. glomerata plants. O. miliacea showed a higher root biomass production and water consumption (availability) as a consequence of VAM infection. Those data suggests that VAM inoculation at the nursery could be a useful practice to enhance plant performance during the initial developmental stages under field conditions in O. miliacea. A. mauritanica biomass production and quality was compared to that of switchgrass and giant reed, showing relatively higher
biomass production than switchgrass but lower biomass quality parameters than both giant reed and switchgrass.

Task 3.2 Good agricultural practices for selected perennial grasses.

Sowing/planting strategies

Sowing strategies were conducted with switchgrass since it is a fertile species which can be reproduced by seeds. However, during its establishment timely and effective weed control strategies are needed, as well as careful soil tillage and a firm seedbed to achieve a successful stand. On the other hand, for perennial grasses unable to produce fertile seeds, such as giant reed or the triploid hybrid Miscanthus x giganteus, the agamic propagation seems to be the only method for establishing. A dramatic drawback is the high cost of vegetative propagation (rhizomes and micropropagated plants), low plant density at establishment and low yield at first harvest. The propagation via stem-cuttings represents a more economic and environmentally friendly method than rhizome propagation. This method does not require the considerable work involved in digging up, breaking apart and replanting rhizomes, as the propagation material is the aboveground biomass; it also makes the multiplication rate several orders of magnitude greater than that achievable through rhizome propagation.

To examine the effectiveness and advantages of hydro-seeding of switchgrass compared to conventional seeding, two consecutive trials (in 2013 and 2014) were performed at the Experimental Farm of UNIBO, Italy (44° 54' N; 11° 40' E; 32 m a.s.l.). In 2013, hydro- and conventional mechanical (MS, pneumatic disk seeder, Damax, Italy) seedlings were factorially combined with three soil tillage methods: conventional (CT, ploughing 40 cm depth then harrowing), minimum (MT, combined sub-soiler and discharrowing 20 cm depth) and no-tillage (NT, sod seeding). In 2014, only two tillage methods (MT and NT) were evaluated in combination with three hydro-seeding methodologies. In 2013 (trial 1), a significant interaction sowing type x tillage (P≤0.05) was found on switchgrass emergence: CS/NT provided the highest emergence rate, and HS/NT the lowest. HS/MT enhanced switchgrass emergence, but the amount of weeds was also considerable. The number of weeds was only affected by tillage (P≤0.05): CT showed a considerably higher amount of weeds (+400 %) than NT and MT. In 2014 (trial 2), CS showed a faster emergence rate (data not shown), as well as a higher number of seedlings. No significant effect of HS mixture was found on seedling emergence. Likewise, no significant differences emerged between MT and NT on seedling number and emergence rate. In contrast, weed emergence significantly differed between NT and MT, the latter showing fewer weeds (P≤0.05). The aboveground dry biomass (AGB) was similar in all treatments, averaging 4.7 Mg ha−1; HS3/MT showed the highest AGB (5.5 Mg ha−1). Weed biomass was similar in all treatments. Switchgrass re-growth (trial 2 only) was measured as the number of tillers in spring 2015. Significant differences (P≤ 0.01) in tiller number were found between seeding techniques (HS and CS) and between seedbed preparation methods (MT and NT): CS showed a higher number of tillers than HS (900 vs. 588 tillers m−2, respectively), while MT showed a higher number of tillers than NT (734 vs. 598 tillers m−2, respectively). Soil tillage effects on weed population were still detectable in the second year: NT showed a higher presence of weeds than MT (P≤0.05).

Giant reed rhizome (RP) vs stem cutting propagation (SP) was carried out in a field trial in Greece (CRES). At the first harvest (2013), the DMY was significantly higher in RP than SP (P≤0.01). Both stem height and stem density increased at the second harvest (2014); however, no significant differences were found between RP and SP. DMY increased from the first to the second harvest, 5.6-fold in RP and 5.8-fold in SP with no significant differences. At the third harvest (2015), a marginal significance (P≤0.06) was detected in DMY, with RP higher than SP (54.7 and 30.8 t ha−1, respectively). Further to the field trial, propagation of giant reed by “single-node segments” was carried out at UNICT, which provided important and novel information. Out of several factors tested (node position, air temperature, hormone treatment), minimum air temperature (i.e. transplanting time) was the main determinant for rooting development. The highest rooting rate was registered when the condition of maximum and minimum air temperatures were 28 °C and 24 °C, respectively. When transplanting time corresponded to minimum air temperatures below 10 °C, no rooting was observed (e.g. November transplanting time). When the rooting rates of basal, median and apical node cuttings were plotted against the mean minimum (Tmin) or mean maximum air temperatures (Tmax) during the rooting period, significant correlations were obtained between Tmin and median nodes (r=0.84*); however, no correlations were found with basal ones. The apical nodes exhibited the highest sensitivity to Tmin (r=0.87**) and to a lesser extent with the Tmax (r=0.79*). A threshold value of temperature for rooting was calculated at the abscissa intercept (y=0). Base temperature for rooting was 10.4 °C for the median (y=5.52x-48.04; R2=0.71) and 10.2 °C for the apical nodes (y=5.95x-60.56; R2=0.75). Nodes from the basal part of the stem showed, averaged across transplanting times, the highest rooting rate (65.0 %) as compared with the other two node positions (45.1 %).
and 39.8 % for median and apical, respectively). NAA and IBA pretreatments enhanced rooting rates 1.20- to 1.29-fold in warm transplanting periods and 7.95- to 5.96-fold in cold one.

Field establishment by Miscanthus stem propagation from stem-bud activation (in connection with WP1) carried out in Hungary (PRIMUS) showed that plots established in late July had significantly greater stem density (23.3 m−2) than those established in early July, August, early and late September. The same trend was also confirmed in the second and third year. The AGB showed a similar pattern, with the transplanting time effect being significant (P≤0.01) and biomass production of 15.8 and 27.5 t ha−1 (second and third harvest, respectively) at the transplant in late July. The time of stem transplant, influenced shoot density and, hence, biomass yield. An effect of air temperature was observed, with higher minimum temperatures enhancing new shoot emission, suggesting that temperatures close to 20 °C would be ideal for the successful establishment of activated stem cuttings. An important factor to take into consideration is, however, the availability of water.

Growing under salinity and water stress
This pot trial studying salinity and water effect of giant reed (in connection with WP1) showed that clone “Agrigento” seems to be suitable for cropping in Mediterranean areas under water stress (WS) conditions, due its smaller decrease in net photosynthesis (Asat), relative water content (RWC) and green leaf area (gLA). Useful characteristics under WS were also observed for parameters such as chlorophyll content and gLA, among others, with this clone having the lowest stress susceptibility index (SSI) value. In contrast, clone Martinensis seems to be the most salt tolerant clone, making it suitable for cultivation in marginal lands where salinity may predominate. In order to have a successful and constant performance and obtain a substantial biomass yield, which is the main objective of growing perennial grasses, we propose clone Martinensis and Piccoplant as the most suitable clones for growing under both stress conditions for biomass production. This result has important implications for the agronomy of these plants in marginal lands. Fondachello seems to be the most tolerant to increasing salinity levels, followed by Cefalú and Licata, and this confirmed the low tolerance of Agrigento and Tortorici to salinity. However, considering the projected future effects of climate change, it is important to focus on giant reed clones that perform well under both stresses.

Field trial of Saccharum spontaneum spp. aegyptiacum grown under rainfed, 50 or 100% ETm restitution indicated that this species possesses a range of agronomically desirable traits of biomass crop: C4 plant, high biomass yield, active assimilation rates during drought-stress periods and able to use water efficiently. Indeed, this species native from Northern coasts of Africa, looks well adapted to the drought environment of Southern Europe. Soil water availability significantly increased aboveground biomass yield and stomatal conductance, allowing for more CO2 assimilation but raising also the water loss via transpiration. However, damage due to low water availability (soil water close to wilting point) was avoided by reducing stomatal conductance and the utilization of water was improved. An asymptotic non-linear model showed that biomass production as function of water used by the crop increased almost linearly up to 750 mm of water to reach a plateau above 1000 mm. Maximum biomass yield of 37.86 t DM ha−1 were reached at 1150 mm crop water use. On the other hand, a linear negative correlation was found between water use efficiency (WUE) and crop water use. The lower the crop water use the higher the WUE. WUE ranged from 5.91 to 3.23 g L−1 as crop water use increased from 390.1 to 1148.2 mm.

In Miscanthus spp. grown in field in rainfed or 100% ETm restitution, differences between accessions were observed, both in terms of physiological behavior and biomass yield. Accession M6 and M7 confirmed the drought tolerance shown during the summer time even at harvest, when no differences between well-watered and rainfed conditions were registered. As compared to Miscanthus x giganteus (M10, the reference species), an even higher biomass yield was achieved in M6 and M7 in both treatments, suggesting further studies on these accessions in environment characterized by drought conditions, as those of south Mediterranean area. Other accessions, as M1, M2, M3 and M4, would be worth to be further studied by using mild water conditions due to their extremely high biomass yield in moist conditions. Finally, M13 and M14 could be also used in breeding programs due to their strong drought tolerance, even if biomass yield did not resulted as high as the accessions above.

Switchgrass (Alamo and Kanlow), sowing row distances (35 and 70 cm) and irrigation rates (rainfed, 50 and 100% ETm) were compared. At the establishment year the achieved dry yields were 7.63 t/ha, averaged all factors. Switchgrass yields were significantly increased (more than three times higher) in the second year (24.92 t/ha) and remained almost the same in the third year (24.29 t/ha). In all years, the variety Kanlow was more productive than Alamo (25.35 vs 23.23 t/ha, 3rd year). The plots that had larger distances between the rows were more productive compared to the narrow rows plots and this superiority was stronger at the end of the third growing season (27.49 vs. 22.85 t/ha). It was found that when the irrigation
rate was increased the yields were also increased with differences between rainfed and 50% ETm. The irrigation effect on the yields was stronger in the second year than the third due to lower rainfalls. No differences were found between 50 and 100% ETm.

Assessment of saline lands in Spain available for growing perennial species.

UPM identified the marginal lands that could be irrigated with saline waters for cropping tolerant-energy crops (Arundo donax L.) in mainland of Spain and assess their surface. To this purpose, a GIS analysis was conducted using geodatabases related to saline areas, pedo-climatic conditions, Arundo water requirements, agricultural land availability and regulated water provision. The spatial GIS analysis performed in this study to identify saline and prone-saline agricultural areas available for growing giant reed showed that the irrigated agricultural area of Spain that met the above mentioned criteria was mainly located in the southeast Spain and represented 34,412 ha plus 61 ha of abandoned irrigated agricultural lands. In the identified areas the potential yield of giant reed was estimated in the range of 12-22 t DM ha. According to the GIS approach, the biomass potential production might reach up to 597,338 t DM year.

Homogeneous feedstock test

In southern Mediterranean environments, a side-by-side field trial of giant reed and miscanthus for three subsequent growing seasons showed that giant reed outperformed miscanthus in terms of biomass yield. When two harvest times were compared (autumn vs winter harvest), winter harvest increased biomass yield and quality, representing a more suitable feedstocks in both thermochemical (moisture and ash content) and biochemical conversions (NDF, ADF and ADL content) for both species. In switchgrass two harvest times were also tested in two subsequent growing seasons; end of November and middle of January. In both growing seasons (2013-4 & 2014-5) when the harvesting was delayed the moisture content was decreased. Regarding the yields different trends were recorded; in the growing season 2013-4 when the harvesting was delayed the dry yields declined, while in the growing season 2014-5 the dry yields increased. Further research is needed to determine the best harvesting time of switchgrass. The age of the plantation as well as the specific climatic conditions in each growing season could play an important role on the appropriate harvesting time.

3.4.3 Impact on other Work Packages

The research on endemic grasses highlighted that there is a great potential among the Mediterranean flora to provide new species for bioenergy production or ecosystem services (e.g. erosion control, carbon sequestration, soil fertility). Saccharum spontaneum spp. aegyptiacum, C4 crop, native from the northern coast of Africa (Egypt) and widespread in Sicily, showed to be the best ideotype in terms of biomass yield and water use efficiency, with values comparable to those of known perennial grasses, as giant reed, miscanthus and switchgrass. Piptatherum miliacium accessions differed for quality traits of biomass, and the existence of this variability can be advantageously used to set up a program of genotype selection aiming at obtaining plants suitable for the several bioenergy uses. Furthermore, this species can be propagated by seed. For species showing slow growth rate at the establishment (common for perennial grasses in general) VAM inoculation at the nursery could be a useful practice to enhance plant performance during the initial developmental stages under field conditions, as found for Oryzopsis miliacea. These results can have a positive impact for further studies on environmental sustainability, but out the horizon of the project.

Results of task 3.2 were used as basis for WP7, where an integrated assessment of sustainability was carried out. In particular, biomass yield and other traits of selected perennial grasses under stress conditions were useful values to determine yield reduction in marginal simulated lands (e.g. salt and drought stress conditions). Other subtasks as sowing and planting strategies and homogeneous feedstock tests were determinant for establishment and harvesting time in WP4, where a farm scale productivity of perennial grasses was carried out, as well as for WP5 for the technological bioconversion. Furthermore, the spatial GIS analysis performed to identify saline and prone-saline agricultural areas available for growing giant reed in Spain might serve as starting point for mapping marginal lands in Europe for perennial and other industrial crops.

3.4.4 WP3 Deliverables

Overall WP3 results are available on the deliverables (D3.1-D3.12) submitted throughout the 4-year project.

3.5 WP4 Farm-scale productivity of perennial grasses on marginal lands

3.5.1 Aim

The general objective of the WP4 is the evaluation of (i) different intercropping on marginal land, (ii) the productivity in
marginal large fields, (iii) the long term productivity of existing large-scale plantation, (iv) the reduction of biomass during harvesting and storage and the (v) subsequent logistics and preprocessing.

This WP is under the coordination of partner CRES; contribution is given also by UNICT, UNIBO, CNR-ISPAAM, UPM, CRA-ING, SPAPPERRI, CRDT and 2ZK.

3.5.2 Project Scientific and Technical Results

Task 4.1 Intercropping on marginal land

The objective of task 4.1 is to develop a sustainable biomass production in drought-prone environments using environmental-friendly agronomic techniques specifically in the first year of biomass crops, when biomass plants show a low yield and a low ground cover.

Grass (Arundo donax and Miscanthus x giganteus) and legume (Medicago sativa or Trifolium alexandrinum) were mixed, combined with nitrogen fertilization (0 or 80 kg N ha-1). The trial was done in Greece, Sardinia and Sicily Islands. It was observed that perennial grasses show a low yielding capacity at the first year, since they have to develop their rhizomes and assure a good establishment for the rest of their growing life. The trial concluded that giant reed and miscanthus had a better growth and yields when cultivated alone, compared to their growth in the intercropped plots. Growing legume in the perennials’ plots caused a 30% reduction in the plant height whereas the number of stems per square meter dropped to one third. As for the yields, there was also a serious reduction in the intercropped plots, with giant reed yields dropping to a quarter and miscanthus to a third compared to the pure stands. It seems that growing a legume within the giant reed and miscanthus plots instead of contributing to nitrogen fixation and energy use efficiency it actually increased the competition between the intercropped crops for natural resources (light, available soil water and nutrients) decreasing thus the final yields of both crops. It was noticed that in the second harvest of Medicago plants in mid-August, when the perennial grasses are still at their growth stage but severely stressed by the high temperatures and lack of irrigation water, yields of Medicago in the intercropped plots were higher by 17-20% than yields in the alfalfa pure stands. The presence of additional nitrogen fertilization did not affect giant reed yields; on the contrary yields were higher in the non-fertilised plots compared to fertilized ones both in the pure stands and the intercropped plots. The opposite trend was noticed though for miscanthus. It is evident that further agronomic research is required in order to reach sound conclusions on the interaction of perennial grasses and legume crops during the establishment period of the perennials. A second harvest of the perennials will be carried out in January 2016 to check if the recorded trends persist.

Task 4.2 Productivity in marginal large fields

The objective of this task is to establish promising perennial grasses in rainfed marginal lands in order to evaluate their performances at large scale field level under low input conditions. Multilocational performance studies in marginal lands will evaluate the most promising genotypes of giant reed, miscanthus and switchgrass with respect of water utilization efficiency and non-invasiveness.

In a 0.2 ha field trial in Greece, giant reed and switchgrass variety Bo Master were planted. Yield of giant reed in rainfed conditions were about 25 t DM ha-1, though moisture content in the third growing period was lower (37%) compared to the second growing period (43%). In switchgrass, yields remained at similar levels (15 t DM ha-1 in 2015 compared to 13 t DM ha-1 in 2014), though moisture content in the third growing period was considerable lower (22%) compared to the second growing period (36%).

Five-ha were planted with giant reed in South-East Sicily in complete absence of input supply (no fertilization, irrigation, pesticide and herbicide). Furthermore, minimum tillage was also applied for this perennial. At first harvest, biomass yield was very low (1.8 t DM ha-1) due to strong weed competition; the dry matter yield increased at second harvest to 8.4±2.1 t ha-1 and also at the third one, 12.1±2.9 t ha-1. As the crop grow in rainfall regime, it is worth to note that rainfall was lower and badly distributed than the historical trend of the area in the three growing seasons. A second trial was conducted in a sloping area (26-28% slope) in the inland Sicily (in connection with WP6, erosion trial) in complete absence of input supply. Plot area was 320 m2. In these marginal conditions, giant reed produced less than 10 t DM ha-1 in three subsequent harvest. Saccharum spontaneum produced 4.3±1.1 t DM ha-1 at the third harvest, while cardoon was able to reach 5.7±0.9 at the first year harvest, showing a great potential under these conditions.

In a 5-ha with a prevailing northwest to south slope orientation switchgrass (cv. Alamo) was sown in 2002 in north Italy. Produced biomass was annually mechanically harvested and bailed at the end of each growing season (late September to
October. Past results showed that switchgrass average productivity in these conditions (2002-2007) was around 7 t ha⁻¹; however, yield abruptly changed across the field depending on enormous spatial variability of soil properties. The growth of switchgrass in the last year (2015) appeared impaired, probably considering the marginality of this field the stand is approaching the end of its productive lifespan. The weed presence in some areas of the field was so high to prevent the proper growth of switchgrass, even during its maximum development stage (summer months). The last harvesting (2015) just confirmed what monitored during crop growth and the negative trend on productivity highlighted since 2012. The average biomass yield of the field did not exceed 4 t DM ha⁻¹, the lowest value since establishment.

Task 4.3 Long term productivity of existing large-scale plantation

The objective of this task is to determine long term yields of existing large-scale plantations. This activity will be based on the gained experiences from several previous and ongoing projects in which large scale experimental fields of perennial grasses (e.g. switchgrass, miscanthus, giant reed and cardoon) were established by the partners involved in this task. Seventeen-year dry biomass yields of switchgrass ecotypes grown in Greece showed that lowlands and uplands performed similarly in terms of biomass yield, with the exception of the second year in which lowlands outperformed uplands (P≤0.05). Despite similar biomass yields of lowlands and uplands (10.0 and 9.8 t DM ha−1), the coefficient of variation (CV), which accounts for yield stability, was clearly higher in the uplands (32 vs. 24 % for lowland ecotypes). Miscanthus and giant reed were compared in southern Italy. Miscanthus required a shorter period than giant reed to reach ceiling yields; however, giant reed showed slightly higher potential yield in the long term. It is worth noting that, unlike giant reed, miscanthus was irrigated for three consecutive years, which explains the faster yield ceiling of miscanthus (third year) compared to giant reed (seventh year). After yield ceiling, relatively stable yields were observed in giant reed for 16 years; miscanthus showed a significant decrease of biomass yield after 5 years, then fluctuating yields for 14 years before a visible decline of productivity. Mean annual biomass production of miscanthus and giant reed were 13.3 and 15.7 t DM ha⁻¹, respectively. Yield stability was slightly higher in giant reed than miscanthus (CV 28 and 31 %, respectively). Side-by-side comparison of switchgrass (ecotype Alamo) and giant reed was conducted in north Italy. Aside from the establishment year, in which productivity of switchgrass was clearly higher than giant reed (6.2 and 2.4 t DM ha⁻¹), two periods could be distinguished: (i) year 2 to 7, in which switchgrass and giant reed produced similar amount of biomass; (ii) year 8 to 11, in which giant reed clearly exceeded switchgrass (about 50 % more biomass per year). Thus, in the long term, giant reed resulted in a clearly higher productivity than switchgrass (21.2 and 13.6 t DM ha⁻¹ year⁻¹, respectively), while yield stability of the two crops was quite similar (CV of 34 and 37 % for giant reed and switchgrass, respectively).

Studies like this can greatly contribute to a more comprehensive view of the real potential of these crops in Europe. Long-term yields of switchgrass, giant reed and miscanthus grown in the Mediterranean basin under low input management were highly variable across years and locations. Generally, giant reed and switchgrass were the highest and lowest yielding crops, respectively. Compared with switchgrass and miscanthus, giant reed also showed lower yield variability over time and across locations. Such variability may entail practical implications for planning and developing new value chains.

Task 4.4 Reducing biomass losses during harvesting & storage

This task aims at the verification of the local parameters that involve the crops logistics, including the use of commercial harvesters, equipment for the storage and logistic aspects at demonstrative tests in different Regions of southern Europe to check the efficiency of the agricultural machineries under different operating conditions. The research studied the logistics to improve harvest and biomass density in order to decrease transport costs and reduce losses and metabolite degradation during storage.

Mechanical harvest of cardoon (Cynara cardunculus) in a demonstrative field trial of 8-ha with slopes ranging from 0 to 40%, were conducted in Spain (UPM). Plant growth was influenced by the mixed effect of slope and drought, where land slope was the predominant factor. The trend observed was that the lower the land slope, the higher the biomass production. Accordingly, the height of the canopy varied, this fact was of importance for the mechanical harvest of the crop. A wide range of machinery was used for biomass harvesting across the years, showing that it can be undertaken by conventional means. The quality of the stems for solid biofuel purposes was much better than leaves every year; however, the influence of the leaf fraction on the whole biomass quality was low because it represented only a small proportion of the harvested biomass. Cynara proved to be a very hardy crop, overcoming harsh soil&climate conditions. In order to reduce biomass/seed losses during the mechanical
harvest, the harvester should be able to work in a wide range of plant heights, since this plant trait has been revealed very
dependent of the conditions of the growing season.
CREA-ING and SPAPPERI worked in Italy to evaluate the performance of two systems for harvesting giant reed in sloping land,
where spontaneous plants of giant reed grows alongside the river banks. Two chopping systems were compared: a hydraulic
forestry mulcher with hammers, mounted on the arm of an excavator, and a hydraulic mulcher with knives, on a self-propelled.
The physiological stage (harvest time) influenced the yield, the moisture content and, finally, the dry matter. Although the gap
among the two test was remarkable, the productive data were interestingly high if compared to a dedicated cultivation. The
bulk density of a feedstock is a key factor for the logistic because the total volume affects the number of travels, thus
determining the cost of transportation. The different type of vegetation led to an increase of the bulk density, passing on
average, from 92.6 kg m-3 in February to 113.2 kg m-3 in June when the plants were in full growth. At the same time, the use
of the heading equipped with knives rather than the hammer (on average, 108.6 vs 97.1 kg m-3, respectively) allowed a
substantial improvement of the weights. Despite a different field efficiency, the theoretical field capacity (TFC), the effective
field capacity (EFC) and the work productivity (WP) showed a gradually increase going from the processing in February and
June with the hammers, until the operations in June with knives. The improvement observed for the hammers was small for
TFC and EFC, but remarkable for the WP. For this latter, the different productivity of the plant is responsible of the
multiplicative effect on the final result. The best results showed by the heading with knives for TFC, EFC and WP was due to
the combination of both the performance of the implement the yield of the Arundo in June.
The evaluation of bulk collection systems as an alternative to bailing was conducted. The role of counter-blades position
showed a weak effect of the shifting of particle size distribution towards the longer classes, increasing just 2.5% the proportion
of pieces longer than 12.5 mm. However, it is important to note that compared to previous experiences made with a self-
propelled forage harvester, there was a substantial increase of the proportion of the fractions belonging to the longer classes
(25-50 and 50-75 mm). In summary, the position of counter-blades may give better results if coupled to the regulation of the
rotor speed that appears a factor more crucial for the pursued objective.
CREA-ING, in collaboration with the SPAPPERI Ltd, developed a semi-mounted prototype for the harvesting of Arundo aiming at
to obtain stem sections longer than 1-3 cm. The machine has a working width of 2.8 m and is composed of two main parts: i) a
cutting system coupled to two vertical rotors conveying the plants towards the in-feed rollers; ii) a disc chipper rotating on
horizontal axis equipped with two knives in radial position. The two components are connected by two counter-rotating
toothed rollers having the function of picking up the plants to convey at the disc chipper. The chipping system is based on the
typology of the forestry chippers. The positions of the main disc holders and the blade holder have been modified to enlarge
the cutting opening and favor the lengthening of the cut. The functional scheme foresees the use of a tractor with reversible
drive and a tractor-trailer combination moving parallel to the machine where the chips are blown by a swiveling goose-neck.
When loaded, the tractor-trailed unit moves to a collection point where the feedstock is discharged.
Degradation during storage
The study of the principal polymeric components of Arundo donax mechanically harvested showed that lignin content was
little affected by storage respect cellulose in all treatments. However, taking into account the dry matter losses, CRA-ING and
SPAPPERI observed that also the lignin was in part affected by degradation. This fact was probably due to the presence of
ligninolitic fungi. Considering the potential use of Arundo donax biomass for bio-ethanol and biogas production, an interesting
observation is that the amount of cellulose preserved in unventilated bins was the 10% more respect to the traditional
method (open-air piles). Indeed, without the use of electricity, part of the biomass fraction convertible into energy can be
preserved in any case adopting bins and probably other similar solutions. The use of ventilated bins allowed preserving 40%
more cellulose than traditional storage method. This will reflect in energy performance of the biomass much higher during
conversion into bio-ethanol or biogas. However, further studies are still needed to verify the convenience of the method in
economic terms, i.e. if the costs of ventilation are lower than the cost of energy obtained. In general, the treatment that allows
preserving more lignin and cellulose was the ventilated treatment, while the worse storage performances were exhibited by
open-air piles. On the other hand, the study has pointed out that the amount of cellulose degraded may result in any case
very high, considering also the relatively short storage period. A possible explanation is that the cellulose, because of its
chemical composition, is much degradable if exposed to microorganisms. Indeed, the chopping of the whole plants determined
the breakage of many cells, increasing dramatically the surface exposed to degrading agents present in the air and on the
soil. In addition, the material was stored in late spring/early summer in Mediterranean climate, within a range of temperatures that typically favour microbial growth. In the case of energy produced from combustion, the study of moisture content and dry matter losses showed that ventilation has remarkably influenced the energy potential of the biomass stored, allowing efficient drying and limiting degradation. The study of fuel quality revealed very low changes in energy characteristics of the biomass, but at the same time confirmed what is indicated in the available literature, i.e. a high presence of ash and the low ash fusion temperature point.

**Task 4.5 Form harvest, logistics and pre-processing**

In this task processing (to pellets) configurations were established for perennial grasses, keeping the mobile densification unit (MicroMill or CargoMill) as key feature for its economical development. Several benefits were demonstrated about a mobile system (CargoMill) vs. a stationary system about the pellets run and the final recommendations. Despite the energy costs of the pelletizing process, this step is remaining mandatory for further application either in energy or in non-energy application. The benefits are, among others, triggered by a reduction of the carried volume (kgs/m3) and subsequent CO2 emissions (from diesel) and with a reduced volume, a reduction of the transport tax, a rising concern across Europe especially for the transport companies. Nevertheless, the main identified barrier is still the financing. The financing community is relying on mature technology (technology readiness level of 6 and above).

The recommended self-propelled with embedded drying and densification unit, even if mounted with existing and mature technologies (TRL 7-9) is not yet in operations and at least 2000 hours of operations will be necessary to prove its full operationability.

For a SME, like 2ZK, the permanent same barrier of the financing is remaining as a major constraint for the development of innovative operational model. Workable business model are oriented to energy production (supported by local feed in tariff) and/or specific business model for the production of one ready product.

In the last months of the OPTIMA project, more funding solutions were tested either at the regional level (Wallonia) and at the EU level (SME Instrument, Fast Track to Innovation). At the regional level (Wallonia), the efforts of the regional authorities are stressed on IT and internet developments. Therefore, without any domestic market, the development of efforts will be very tough. In the meanwhile (2014-2015), several biobased SME’s (flagship) with operations in Wallonia have stopped recently their activity e.g. in the biogas. Following these bankruptcies or liquidations the regional authorities and the local public investment companies are very cautious toward any biobased project and in particularly about the upstream supply chain.

On the other hand, we could rely in Wallonia on two major corporations: Total Petrochemicals (Feluy) and Solvay which haven’t so far answered to the inquiries. The contact between SME’s and large corporations is still not very easy. It should be noticed that some countries are performing well at the regional level. NL and some counties are really committed to shift from a carbon economy to a low carbon or biobased economy. The results have started to be visible with efficient clusters of SME’s engaged in the biobased economy. The County of Brabant is a typical example. Somehow, BTG (based in Enschede, County of Overijssel) has, probably, also had the benefit of such commitment.

At the EU level, several applications were prepared and submitted for project with transnational partners.

3.5.3 Impact on other Work Packages

These findings were used for WP7 where the integrated assessment of sustainability was carried out. Harvesting and subsequent logistic optimization might impact the final biomass yield and calculations. For instance, not recovered biomass during mechanical harvesting was found considerable revealing that there is still great possibility to improve the actual yield through an appropriate set up of baling machines and timely harvesting.

Autumn harvesting appears a better option for switchgrass compared to killing frost harvesting for North Mediterranean sites due to winter snow and unfavorable weather conditions that may occur in winter. Nonetheless, it would be important to understand the effect of early harvesting period (autumn) on plant life span as mineral translocation will be interrupted at the likely expense of long term rhizome vigor.

On the other hand, in south Mediterranean, winter harvest seems to be the best option to harvest giant reed and miscanthus for both quantity and quality of the biomass.
Long term studies can greatly contribute to a more comprehensive view of the real potential of these crops in Europe. Long-term yields of switchgrass, giant reed and miscanthus grown in the Mediterranean basin under low input management were highly variable across years and locations. Generally, giant reed and switchgrass were the highest and lowest yielding crops, respectively. Compared with switchgrass and miscanthus, giant reed also showed lower yield variability over time and across locations. Such variability may entail practical implications for planning and developing new value chains.

3.5.4 WP4 Deliverables
Overall WP4 results are available on the deliverables (D4.1-D4.21) submitted throughout the 4-year project.

3.6 WP5 Energy products and plant derived biomaterials

3.6.1 Aim
WP5 will assess the various possibilities to use the perennial grasses for energy and materials. New innovative technologies will be tested for the various crops, in order to be able to determine the most promising applications for the crops. The assessment of the local downstream biomass market and the maturity level will be also carried out.

This WP was under the coordination of BTG; contribution was given also by 2ZK, CRES, CRDT.

3.6.2 Project Scientific and Technical Results
Task 5.1 Determination of the characteristics for energy products of the perennial crops
During the first year, eight samples were provided by UNICT from giant reed (3), miscanthus (2) and Saccharum (3). The samples were from the same location and cultivation techniques. BTG pretreated the samples and tested them according to the DoW. In the second year, samples of the cultivated crops were made available by the partners and characteristics that influence the suitability of the crops to be used for energy were determined by both BTG and CRES. The results have been compared with literature and with previous projects. The results showed a great variability in the samples. In all cases there was a high ash content. Most of the samples showed low ash melting temperatures (IDT’s) and high Nitrogen concentrations. Due to Cl, S and K there is a high corrosion potential, which is also reported in literature. Delayed harvest and leaching have a positive effect on the characteristics for energy use.

Task 5.2: Technology assessment of promising pathways for conversion into energy products and bio-products
Based on the results of Task 5.1 a technology assessment was made to select the most promising pathways. The pathways include the whole chain from pretreatment and logistics of the harvested crop to conversion into useful energy and products. Selection has been made based on the fuel properties of the selected crops, the state of the art of the conversion technologies, and taking into account promising innovative conversion pathways that could produce higher added value products. In this way seven pathways have been selected. The pathways also include pyrolysis and torrefaction, as in Task 5.3 pilot tests will be executed with these technologies in the next period of the project. Special attention was paid to pelletising as a pretreatment step. The handling and quality of the feedstock is improved, but CO2-emissions and costs will rise.

Task 5.3 Pilot tests with secondary conversion technologies: pyrolysis and torrefaction
Based on the results of the previous tasks, Arundo Donax and Miscanthus samples were selected for the torrefaction and pyrolysis experiments. The tests were carried out in experimental facilities available at BTG. The samples for the pyrolysis and torrefaction tests have been made available by UNICT. The aim of the tests was to get an impression of the suitability of the crops for the aforementioned pre-treatment options to make them suitable for the application in biorefinery processes. Torrefaction and pyrolysis are considered as promising pretreatment and conversion technologies for biorefineries. For both processes, demonstration plants are currently under construction at full commercial scale. However, up till now mainly woody biomass is used as feedstock and only limited data is available for herbaceous crops like Arundo and Miscanthus. For the torrefaction experiments the mass yield was a bit higher for Miscanthus (75 w% versus 69 wt%), while the energy yield of Arundo material was higher (90% versus 87%). Such mass yields are comparable to the ranges usually found for woody biomass (70-80 wt%). Based on the limited tests, both crops are considered suitable for conversion via torrefaction. In the pyrolysis test the Miscanthus material yielded more liquid than Arundo (63 w% versus 55w%), with higher energy and carbon yield as well. Typically, pyrolysis of wood yield 60-65% liquids which is comparable to Miscanthus. It is expected that the higher yield of Miscanthus compared to Arundo is also caused by the lower ash content of the Miscanthus feedstock (3.4 wt% versus 5.6 wt%). Partly because the ash cannot be converted into pyrolysis oil, but even more due to the catalytic effect ash components play in the pyrolysis process. In general, higher ash contents typically lead to lower pyrolysis liquid yields.
Typically wood contains less ash and results in high pyrolysis liquids yields or in torrefaction char yields. When the ash content rises, the pyrolysis liquid yields and torrefaction char yields decline. This is in line with the Arundo and Miscanthus tests. The torrefaction test showed that the nitrogen from the feedstock remains in the char. The torrefaction liquid did not contain nitrogen. In the pyrolysis process the nitrogen was divided both into the pyrolysis oil liquid and the char. For the Miscanthus sample (with 0.5 wt% nitrogen) the nitrogen content in the liquid was 0.4 wt% and 0.6% in the char. For Arundo (1.2 wt% nitrogen) the nitrogen content in the liquid was 1.1 wt% and 1.2% in the char.

It is emphasized that the above conclusions are based on a limited number of tests that are planned in the OPTIMA project, due to budgetary limitations. To be able to draw more general and reliable conclusions, additional tests are required in the future with a larger number of samples and under varying reaction conditions.

Task 5.4 Biobased products

In task 5.4 the possibility of using perennial crops for the production of sugars for fuels and chemicals, as well as for non energy applications has been assessed. CDRT-IITD has researched the possibility to use perennial biomass grasses for sugars production for fuels and chemicals. At start extensive literature was collected and reviewed related to the various pretreatment methods given to the cellulosic biomass, hydrolysis and fermentation process. Various factors effecting reducing sugar production during pretreatment method have been identified.

2ZK has further focused on the possibility of using perennial crops for the production of sugars for fuels and chemicals, as well as for defined non energy applications. Special focus has been on the testing of perennial crops for (possible) use as animal bedding, insulation materials and packaging materials, biocomposites, sugars C5-C6. In house tests were performed by 2ZK with Miscanthus for animal bedding applications (and benchmarked with giant reed and wood pellets). The focus of the tests was oriented for market applications after the first conversion step (i.e. the densification process). Subsequently other conversion routes can be applied that require a second conversion process e.g. conversion into biomethanol and C5-C6 sugars. In addition to the experimental work also results were used from the literature review that was executed. New chains needs to secure revenues and therefore must identify immediate markets with a low level of regulations and a simple preparation or conversion process: animal bedding mulching are very convenient market. The main restriction that must be highlighted is related to the hygiene standards in place per country or at the EU wide level. Such standards and barriers are often unexpected (bird flu) and will ban raw material from the bedding and mulching markets (where no second conversion, thermal or chemical process, is necessary).

Task 5.5 Market analysis and regulatory framework for bioenergy and bio-based products in Europe

In the previous periods already an extensive market study was initiated in order to determine possibilities for using the fermentable sugars in perennial crops. Market volumes were listed as well as prominent industries that produce bioethanol from different feedstocks. Also current experiences with the production of bioethanol from grasses were included, showing results with different pretreatment methods. The regulatory framework for bioenergy and biobased products in Europe was also covered. The task dedicated to the bioenergy applications (especially applications for biofuels) was performed by CRDT. For more or less sophisticated applications the learning curve was improved in several ways like contacting market players, prediction of market behavior and market price level, and identification of a regulatory framework to get a level playing field. For energy applications, a huge potential was identified by 2ZK especially in Ukraine, as import of natural gas from Russia is uncertain. The use of biomass, including energy crops, for energy purposes in local district heating plants has gained a lot of interest during recent years. Drawback is the restraint of foreign investors due to the unstable political situation in Ukraine. In addition 2ZK engaged many contacts in the fourth reporting period with local municipalities in Belgium (which are member of the Covenant of Mayors) by offering a green heat solution for public buildings. Also, 2ZK has continuously managed intensive contacts with European industries like for example DSM, Bridgestone, Soudal and also with local large corporations (Total Petrochemicals, Solvay). For confidentiality reasons initially the response and cooperation of these industries was relatively low. As a solution therefore a new approach was chosen by means of preparation of a concrete business case about a biorefinery for the conversion of local energy crops into biobased building blocks. This project was subsequently presented to several local stakeholders.

3.6.3 Impact on other Work Packages

Results of task 5.1 on fuel quality confirmed that delayed harvest and nutrient leaching have a positive effect on the characteristics for energy use this is very important for WP3 and WP4 where field trials were carried out.
For each selected pathway the mass and energy balances were attempted. Also greenhouse gas savings were presented for the pathways, relative to the fossil pathways. The mass and energy balances and greenhouse gas saving potentials were main input to WP7 where an integrated assessment is made.

3.6.4 WP5 Deliverables

Overall WP5 results are available on the deliverables (D5.1-D5.8) submitted throughout the 4-year project.

3.7 WP6 Environmental studies on perennial crops

3.7.1 Aim

WP6 exploited specific bottlenecks regarding environmental implications of perennial grasses on marginal lands. Particular attention was paid to the following issues: (i) Assessment of CO2 and water exchange between canopy and atmosphere through micrometeorological “eddy covariance” measurements; (ii) Root development and carbon storing in the soil; (iii) Energy balance; (iv) Phytoremediation; (v) Soil erosion mitigation by perennial grasses; (vi) Environmental Impact Assessment (EIA).

WP6 was under the coordination of UNIBO; contribution was given also by FCT-UNL, PRIMUS, CRDT and UNICT.

3.7.2 Project Scientific and Technical Results

Task 6.1 Assessment of CO2 and water exchange between canopy and atmosphere

To our knowledge, the first experience carried out in Europe using an eddy covariance system to assess CO2/H2O fluxes at canopy level in switchgrass for four consecutive growing seasons was done within the OPTIMA project. Soil is an important sink for the carbon storage in the form of soil organic carbon (SOC) and reduction of CO2 in the atmosphere. The trend of fluxes in the fourth growing season clearly showed the beginning of photosynthesys activity from the middle of March.

Comparing carbon fluxes with the previous year, C sequestration in the last growing season resulted lower due to the scarce precipitation during summer. The Net Ecosystem Exchange (NEE) showed an increase from March to June in 2015 (as negative values), reaching a peak of -2.1 Mg C ha-1 month-1 (June 2015), thereafter NEE decreased. The same trend was observed for the GPP values with a maximum of 3.4 Mg C ha-1 month-1, reached in June. The trend followed the trend of temperatures. The NEE was negative during the whole growing season, meaning that the ecosystem respiration was lower than the C sequestration. The respiration started to be higher than the GPP after 2014 harvesting, the same trend was not yet observable in 2015, as C fluxes after harvesting are still under elaboration. The NEE, GPP and Re cumulative values from harvest 2014 to harvest 2015 were 14.6, 32.7 and 18.2 Mg C ha-1, respectively. Evapotranspiration was also monitored through the eddy covariance technique during the last growing season. Since irrigation was not applied after establishment, the total water contribution was due to only to precipitations. Monthly ETc was found higher in 2014 when compared to 2015 season, except for June, when this tendency was reversed; thereafter ETc rapidly decreased in July and August because of rainfall scarcity. Water availability represents a key factor to achieve stable and high productions in switchgrass. The last growing season was characterized by limited rainfall during summer compared to the previous years. During the last season the soil water content was no able to compensate the water lack and consequently the crop faced a significant stress with consequent reduction of photosynthetic activities. Only after some rainfall carbon fixation was able to reach similar values than the previous years.

Task 6.2 Root development and carbon storage in the soil

FCT-UNL was working on the effects of long-term plantations of Miscanthus x giganteus and Arundo donax on the soil organic matter and soil carbon sequestering in comparison with fallow soils. FCT/UNL results showed that the presence of Miscanthus in the soil contributed to an increment of organic matter by comparison with fallow soils, although this increment was not statistically significant. The same trend was observed for A. donax but, in this case, the increment was statistically significant. Recycled carbon to soil from litter of Miscanthus represents ~3.1-3.9 Mg ha-1 year-1. In the case of A. donax, it represents 2.6 ± 0.4 Mg ha-1 year-1. Carbon sequestration by the root and rhizome system is considerable, representing about 12.5-13.5 Mg ha-1, over the life time of the Miscanthus system, and 21 ± 2 Mg ha-1, in the case of the A. donax system. But this soil-rhizome accumulation can become prejudicial if land use will be changed, by release of the stored carbon.

The effects of perennial grasses (switchgrass at UNIBO) on the carbon storage were evaluated in comparison to typical annual rotation (maize-wheat). Data of aboveground and belowground biomass, SOC and soil fluxes derived from switchgrass stands set in both, Cadriano and Ozzano (North Italy), were used as dataset to calibrate the DAYCENT model, aiming at assessing the carbon sequestration in switchgrass agricultural systems. Interestingly during 2015 growing season, soil respiration was found
higher in the switchgrass field (+ 108%, on average), than in the maize field. This trend was inverted after switchgrass harvesting and maize harvesting + succeeding ploughing (- 64%, on average). It was hypothesized that the higher amount of root biomass in switchgrass (about 5 times greater) than in maize led to a higher autotrophic respiration rate during growing season. At the same time, after harvesting, ploughing, performed only in annual crop rotation field, caused a sudden increase of gas diffusivity leading to high decomposition rates, thus increasing heterotrophic respiration rates. However, it is worth noting that maize was harvested only for grain, thus corn straws were embedded into the soil at ploughing. Switchgrass system seemed to lose less CO2 than the annual cropping system, since the carbon respired through autotrophic processes is directly uptaken from the atmosphere and therefore recycled, while carbon respired through heterotrophic processes is in any case lost by the soil. After calibration and validation, the DAYCENT model was employed to estimate the potential of switchgrass, used as a bioethanol crop, to store soil carbon when grown in the Mediterranean basin. Two scenarios were simulated: 1) cultivation only on marginal lands (1.76 Mha); 2) cultivation on marginal lands + 5% of arable lands, currently used for cereals, (2.97 Mha). The cumulative switchgrass harvested biomass resulted in 184 and 303 Mt, over 15 years of simulated cultivation, under scenario 1 and 2 respectively. SOC storage values were 6.1 and 12.4 Mt, respectively in the two simulated scenarios. Across the study region, mean annual switchgrass biomass yield ranged between 5.6 and 9.4 Mg ha-1, while annual SOC accumulation was 0.02 to 0.62 Mg ha-1. Then, fossil fuels displacement was also estimated and resulted in the sequestration of 54 and 89 Mt of C, i.e. 198 and 327 Mt of equivalent CO2 in the first and second scenario, respectively. In the second scenario, switchgrass ability to store SOC was much more evident. However, a loss of 54 Mt of crop commodities would be caused by switchgrass cultivation on 5% of arable lands with consequent ILUC effects. At this scope the ILUC was calculated by using some results of the JRC (MIRAGE model). ILUC was found however quite low (16%) when compared to environmental benefits as stored SOC.

Task 6.3 Energy balance

The energy balance of the production of Miscanthus based on well-defined boundary limits and experimental data (fuels, fertilizers, machinery, equipment, pesticides, and crop yields) was carried out by FCT-UNL. This analysis, is mainly focused on the cultivation phase, but can be also extended to the transportation, storage and processing phase. Effects on the energy balance due to the use of marginal soils (including contaminated soils with heavy metals) was assessed. Additionally, the energy balance of the production of giant reed in contaminated soils and by using wastewaters were also estimated. The net energy obtained from the production and use of miscanthus grown in marginal soils was dependent on yield level. The lower the yields obtained, the lower the net energy value being obtained. A threshold of just 1 Mg ha-1 year-1 of miscanthus (dry weight) was estimated as being the limit to obtain Output energy = input energy, in the pedo-climatic conditions of Portugal. Additional inputs, needed to improve the yields in marginal soils (e.g. extra N-fertilizer) or resulting from the marginality of the soils (e.g. increased transportation distance), did not present a significant effect on the net energy obtained. However, the energy balance results needed to be complemented with other parameters, such as the nitrogen emissions or the quality of the biomass for combustion, in order to understand the benefits and constraints associated with growing perennial grasses in marginal soils.

Task 6.4 Phytoremediation issues

The potential of perennial grasses, namely miscanthus and giant reed, to phytoremediate contaminated soils and to phytotreat waters and wastewaters was studied in Portugal (FCT-UNL). Giant reed and M. x giganteus can be considered as interesting candidates for Zn phytoextraction, favored by the metal accumulation observed and the high biomass produced. Arundo donax and Miscanthus genotypes showed to be well suited for phytostabilization of heavy metals contamination as these grasses prevented the leaching of heavy metals and groundwater contamination. Moreover, the production of both grasses in the contaminated soils was not affected by heavy metal contamination or was only slightly affected (reduction in productivity of ca 20%). This indicates that the possibility to reduce metals concentration from contaminated soils by means of phytoremediation using these perennials is of great interest and importance to maintain environmental quality standards and to improve soil quality with relative low costs. Additionally, the reduced metals content in the harvestable biomass can be also looked as beneficial, once contamination is not influencing the biomass quality (in terms of the metal content). Still, more studies are necessary to clarify the mechanisms associated with the absorption and translocation of heavy metals in these perennials, its relation with the growing medium and also the interaction of these contaminants with other elements.

Open field capability of switchgrass to survive/grow in heavy metal contaminated soils in India was done by CRDT-IIDT. The
experiment demonstrated that switchgrass perfectly suited the phytostabilization process as it did not allow the heavy metals to translocate into the shoots or aerial biomass. Miscanthus x giganteus and Arundo donax were also evaluated in red mud flooded soils collected in Hungary (PRIMUS) after an alumina plant collapsed. It was found that miscanthus and giant reed were able to start growing in pots containing red mud. The roots and newly developed shoots of miscanthus plants can vigorously grow in pure red mud or red mud layers. Both species can develop in pure red mud in poorly and well-nourished condition. Miscanthus seemed to be better candidate for remediation as this species showed increased tolerance to the tested unfavourable “growth medium”. The observations showed that starting and living capability of Miscanthus and Arundo on red mud would be good enough for use them to grow on and remediate even pure red mud.

Task 6.5 Soil erosion mitigation by perennial grasses

The effects of different cropping systems on erosion mitigation and soil quality have been evaluated in an inland hill of Sicily Island with a slope of 26-28%. Soil erosion is one of the main concern in the Mediterranean area as it causes pollution of water bodies, critical losses of water, nutrients, soil organic matter and soil biota from the natural ecosystems. Results allowed to point out that perennial grasses showed high level of soil losses at the establishment, mainly due to spaced plant density (1 to 2 plant m-2), thereafter soil erosion was significantly reduced by perennial species (Medicago arborea, Saccharum spontaneum and Arundo donax) compared to annual cropping systems and fallow management. Annual crops, which required soil tillage, reduced soil organic matter content while perennial crops reduced soil erosion and nutrient leaching in addition to an overall increase of soil organic matter content.

Task 6.6 Environmental impact assessment

Within task 6.6 an Environmental Impact Assessment (EIA) study was developed and applied by FCT/UNL to the production of perennial energy crops in the Mediterranean, namely switchgrass, miscanthus, giant reed and cardoon as control species. This task addressed site-specific environmental impacts of the OPTIMA pathways. EIA mainly addressed biomass cultivation, but also biomass transportation and biomass processing and use. The results have given input in tasks 7.5 and 7.6. During the 4th year of the project, attention was given to the local environmental impacts derived from the production of perennial crops in marginal soils. The objective was to determine if a biogenic approach, focused on producing perennial grasses on marginal Mediterranean land as feedstock for bioenergy or bio-based products, could reduce greenhouse gas (GHG) emissions without depleting soil nutrients, water resources, or negatively impacting biological and landscape diversity. Additionally, the impacts derived from the production of these grasses in contaminated land and the impacts derived from the use of contaminated wastewaters in the irrigation were also studied.

Production on marginal soils limited the ethical issues associated with competition with food crops and since perennial grasses showed tolerance to soils marginality and high biomass productivity, thus biomass could be economically valorized. Environmental Impact Assessment (EIA) protocols were applied to the production of perennial grasses on marginal Mediterranean land as feedstock for bioenergy or bio-based products. Obtained results showed that the biogenic system had low erosive potential, reduced disturbance of soil properties, and minimal hydrological impacts. Lower tillage and high biomass production supported biological and landscape diversity. Yet, as a recommendation, site-specific factors should be used to appropriately match the specific crop and location. We concluded that producing perennial grasses on marginal Mediterranean land might be feasible and if appropriately managed would lead to limited environmental side-effects.

Bridging perennial grass production with soil phytoremediation represented an in situ possibility for contamination reduction and stabilization, with low costs. Moreover, introduction of a vegetative cover would contribute to the reduction of erosion and lead to organic matter incorporation in the soil, thus restoring soil properties. It would favor biodiversity and occurrence of soil fauna and microfauna, by providing shelter for invertebrates, birds, mammals and higher cover value for wildlife. It would also contribute to the enrichment of landscape values. Even though, yields could be affected by the metal toxicity, energy savings might be reduced or not guaranteed and biomass quality could not match appropriate standards for industrial uses. Other constraints were related with the fact that soil phytoremediation is a very long process. Furthermore, emission of exudates from plant roots might stimulate the leaching of pollutants to waters and ground waters due to mobilization. In conclusion, calculating the balance of pros and cons in cultivating perennial grasses in contaminated lands indicated more environmental benefits than constraints.

3.7.3 Impact on other Work Packages
Results of WP6 were determinant for the integrated assessment of sustainability of WP7.

3.7.4 WP6 Deliverables

Overall WP6 results are available on the deliverables (D6.1-D6.21) submitted throughout the 4-year project.

3.8 WP7 Integrated assessment of sustainability

3.8.1 Aim

WP7 will provide a multi-criteria evaluation of the sustainability of the entire value chain by taking into account technological, environmental, economic and socio-economic aspects. The most sustainable bioenergy and biomaterial pathways based on perennial grasses will be identified. Based on a technological screening, environmental, economic and socio-economic assessment as well as a screening SWOT analysis, the initial selection of pathways will be further refined and optimization potentials will be depicted.

3.8.2 Project Scientific and Technical Results

Within WP7, a so-called ‘integrated assessment of sustainability’ was performed which consists of a series of individual assessments that separately assess the major aspects determining the sustainability of products derived from perennial grasses cultivated on marginal lands in the Mediterranean region. These individual assessments (Tasks 7.2 to 7.5) are based on exactly the same system boundaries which were defined in Task 7.1. The aim was to investigate the potential impacts of the OPTIMA pathways on all major aspects of sustainability (environment, society and economy) one by one, using a set of existing state-of-the-art methodologies. This was supplemented by separate analyses of biomass competition and further aspects such as public perception or regulatory issues. Finally, all sustainability aspects were integrated into an overall sustainability assessment using multi-dimensional comparison metrics and a structured transparent discussion (Task 7.6).

Task 7.1 System description & technological assessment

In collaboration with BTG and 2ZK who provided data on thermochemical conversion pathways, mass and energy balances for the selected pathways were established by IFEU. These pathways also include pre-treatment of the perennial crops to make them suitable as feedstock for the subsequent conversion process into energy and bio-based materials. For each of the different thermochemical pathways, a typical scale (MW energy or ton/yr bio-based product) has been defined. The results of this Task were transferred to Task 7.6.

Task 7.2 Life cycle assessment

During the fourth project year, the main activity was related to the finalisation of the LCA calculations. After some last fine-tunings of the software tool, the environmental performance of 28 main was evaluated. In addition, a large number of sensitivity analyses and alternative scenarios were investigated in order to identify optimisation potentials. The results show that perennial grasses used for stationary heat and power generation can achieve substantial greenhouse gas and non-renewable energy savings at low additional other environmental impacts. Conversion into and use of 2nd generation ethanol, biochar or precursors for biopolymers show mixed results. In all cases it is essential that biomass is harvested at low water content, otherwise a dedicated strategy is necessary to reduce energy-intensive drying as far as possible. Being generic, LCA is not (yet) able to adequately address local environmental impacts such as water use for irrigation which can lead to detrimental impacts, e.g. in the Mediterranean south. It is concluded that perennial grasses grown on marginal land in the Mediterranean provide potentials for climate change mitigation with comparatively low environmental side-effects, especially if several boundary conditions and recommendations are considered. For instance, it has to be ensured that when occupying marginal land (which is not necessarily unused), indirect land use changes are avoided. The results of this Task were transferred to Task 7.6.

Task 7.3 Cost analysis

Economic analysis has shown that financial sustainability of the cultivation of perennial grasses in Europe is related and depends upon EU policies with regard to the security of energy supply and the protection of the environment. In EU today, the cost of delivered biomass from perennial grasses is somewhere between 65 and 75 € per dry ton, which makes it a rather expensive alternative raw material for energy use within the existing framework of energy prices. Fluctuating oil prices and currency exchange rates increase the uncertainty of any economic forecast and raise the risk of economic and financial strategies. Cultivating perennial grasses in marginal lands of South Europe, in spite of the lower cost of land, is less profitable if compared with cultivation in standard agricultural lands with standard agronomic inputs. The savings from low land rent and
moderate inputs are not compensating the significant loss in biomass yield. As a result the perennial grass feedstock cost for conversion technologies is increased at levels that some conversion plants cannot pay. In view of the advancing agricultural land scarcity and anticipated food market fluctuations, Europe is encouraging non-food cultivations in marginal lands. The support given to farmers differs from country to country and at the moment is related to bio-physical characteristics of lands. A more generous support to European farmers is needed to persuade them to cultivate perennial grasses on marginal lands in order to provide feedstock to conversion processes at competitive prices. This will have beneficial environmental effects, especially in electricity generation (which at the moment burns wood pellets from America!). Disregarding any existing subsidization on the biomass value chains, perennial grass pellets can make a profitable pathway, although the significant investment of pelleting plants is not recovered fast enough to stimulate investors. Home heating with wood pellets is quickly spreading in Europe, because, even at current low oil prices, it works out cheaper. In South Europe (with less annual heating needs) the benefit is smaller and the investment in new equipment is paid back in more than five years, discouraging potential users. Biomass transformation into bio-oil, 2G ethanol, bio-gas, can only be competitive if subsidized. However, the mere fact that we consider comparing the economics of their products directly with fossil alternatives, is indicative of the substantial progress that has been achieved in the last years. A few years ago, such technologies and products were still in the “wish” list. Combined heat and power from perennial grasses is only financially sustainable in situations where capacity utilisation is sufficiently high. This makes district heating in the South of Europe less attractive (not economic unless subsidized), since the heating period is only 3-4 months in most regions. However, CHP is financially attractive in industrial applications, hospitals, hotels, etc. where heat is demanded throughout the year. The results of this Task were Task 7.4 Socio-economic studies The socio-economic analysis has been linked to the cost analysis in Task 7.3 and assessed the socio-economic impacts focusing on both quantitative (jobs, direct, indirect and induced) and qualitative parameters (contribution to rural economy, local embedding and proximity to markets). The approach combined qualitative and quantitative assessment and evaluated the impact in two categories, employment effects and social sustainability. The criterion used for employment was job creation and the respective indicators were direct, indirect and induced jobs which were calculated for the under study value chains with the BIOSEM model (Biomass Socio-Economic Multiplier Model model). The jobs calculated in this work are net created jobs (created jobs minus lost jobs due to replaced previous uses of the land). For the social sustainability impact category the criteria and indicators employed in the analysis were: i) contribution to rural economy, ii) local embedding and iii) proximity to markets. The qualitative indicators were grouped as high, moderate, low and the traffic light system was applied. Except of the analysis for the reference value chains, sensitivity analysis for value chain job equivalents is also performed for the following parameters:
- Crop yields: to appreciate the impact of yielding capacity and cropping system in jobs.
- Logistics: for the large CHP, biochar, pyrolysis and 2G ethanol value chains for three cases for logistics are examined: i) 50 km radius, local, ii) 100 km radius, regional & national and iii) biomass trade beyond 100 km radius, within the country and beyond borders. The results of this Task were transferred to Task 7.6.
Task 7.5 SWOT analysis and biomass potentials An analysis on strengths, weaknesses, opportunities, and threats (SWOT) has been performed for the systems defined in Task 7.1. The analysis, where appropriate, covered the three scenarios, i.e. very marginal, marginal and standard, all for the 2020 timeframe and took into account the results of Tasks 7.1 to 7.4. The SWOT analysis has also been supplemented by a separate investigation on the biomass potentials from the under study perennial grasses. The results were further used in Task 7.6. Task 7.6 Integrated assessment of sustainability The main objective was to conduct and finalise the integrated assessment of sustainability, largely following the integrated life cycle sustainability assessment (ILCSA) methodology developed by IFEU (Keller et al. 2015). To better address the discussion on the net benefit of biofuels, bioenergy and bio-based materials from perennial grasses, OPTIMA applied a multi-criteria sustainability assessment of all investigated pathways comparing products from these pathways to conventional reference products. The integrated assessment of sustainability joins and connects results on individual sustainability aspects to give an integrated view on sustainability of the biorefinery concepts. Indicators and results from the assessments of individual sustainability aspects described above (Tasks 7.1 to 7.5 plus Task 6.6) were collected and subjected to a structured, verbal-argumentative discussion. By means of a benchmarking process, front-runner scenarios were identified. It could be shown that
perennial grasses grown on previously abandoned land in the Mediterranean region provide potentials for climate change mitigation and social benefits in rural areas in particular. Abandoned land does not need to be marginal in terms of biophysically inferior properties to entail such benefits, but the achievement of the OPTIMA project is to bring low-quality, previously abandoned land into production by adopting selected crops and agricultural practices. If utilisation options such as efficient stationary energy generation are chosen, benefits can be achieved which are associated with minor other negative environmental impacts. This is a big advantage compared to many other bioenergy pathways, and in some cases even economic profits are attainable.

However, several boundary conditions must be met:
- Only idle (unused) marginal land without high biodiversity value should be cultivated to avoid harmful direct and/or indirect land use changes.
- Irrigation may not contribute to local water shortages with indirect effects on other water users.
- Risks must be managed and shared along the whole added value chain to increase yield stability, reduce production downtimes and limit potential losses for single stakeholders, in particular farmers.
- Several processes from agriculture to biomass use still need to be brought to technical maturity.
- Biomass should be used for efficient, stationary energy generation (as detailed above) until boundary conditions for the assessed innovative use options improve substantially or other, better options are found.

If the above is the case, the use of abandoned marginal land for bioenergy is largely safe from a sustainability perspective. Other assessed use options may also be sustainable in certain settings under altered conditions, which will require further specific analyses. Further optimisation of the cultivation of perennial grasses on marginal land is therefore necessary, but also possible, with promising results. However, the great advantage of perennial crops, resulting from the long plantation lifetimes of 15 years and more, also means that long-term research, development and pilot projects must be carried out and financed.

The specific crop characteristics determine specialty applications, in which cultivation itself may serve environmental protection purposes (erosion protection, decontamination, capturing nutrients). Moreover, under certain conditions and given appropriate technological maturity, economical utilisation options are available even without funding, such as co-incineration in existing biomass-fired combined heat and power stations or pellet-fired central heating systems. These should be utilised in pilot projects. Thus, the cultivation and use of perennial grasses on abandoned marginal land can lead to substantial overall gains in sustainability if promoted and managed properly by politics, stakeholders and developers in science as well as in businesses.

3.8.3 Impact on other Work Packages

No impact to other WPs were generated by the WP7, since this is the output of the RTD WPs, such as WP3&4 (cultivation phases), WP5 (bioconversion) and WP6 (environmental issues) results, which were used as input. The impact of WP7 is discussed below since gives recommendations to scientists, policy makers, industrial players and farmers on the possibility to cultivate and use perennial grasses in marginal land in Mediterranean region.

3.8.4 WP7 Deliverables

Overall WP7 results are available on the deliverables (D7.1-D7.12) submitted throughout the 4-year project.

3.9 WP8 Dissemination

3.9.1 Aim

WP8 devoted to the dissemination of OPTIMA and its main objective is to develop a whole dissemination plan and its main elements will be the development of a website and intranet, one project workshop and one final conference. Additionally other dissemination activities were scheduled such as articles, presentations relevant to the OPTIMA, leaflets, links with relevant ongoing projects and links with twinning actions that have been established in FP7 (EU-Canada and EU-Argentina). CRES was the WP leader with the contribution of the whole consortium.

3.9.2 Project Scientific and Technical Results

Task 8.1 Dissemination plan activities

It was established and discussed at the kick-off meeting which was held in cooperation with the other two FP7 financed projects, OPTIMISC and GRASSMARGINS. The most important elements of this plan were the followings: presentations of the projects findings to relevant Conferences and Symposia; presentations in national and/or European workshops dealing with
the perennial crops; Articles of the project’s findings to relevant conferences and scientific journals; link with the two relevant to the OPTIMA twinning opportunities that have been developed in the view of the FP7; the EU-Canada twinning on biofuels and biomaterials (www.ec-canada.eu) and the EU-Argentina twinning Soil, Plant and Food (www.ec-argentina.eu); links with every relevant previous or ongoing projects regarding the perennial bioenergy crops (such 4FCROPS, BIOENERGY CHAINS, BIOCARD, JATROPHE, CROPS2INDUSTRY, etc.); links with relevant societies or organisations to non-food crops such as “The Association for the Advancement of Industrial Crops – AAIC (www.aaic.org)”. Throughout the 4-year project, a total of 181 activities (oral presentations and posters at national and international conferences, workshops and symposiums, flyers, interview, etc.) were produced. A total of 13 between Master and PhD thesis and 20 peer-reviewed publications in relevant scientific journals, such as Applied Engineering in Agriculture, Italian Journal of Agronomy, International Journal of Ecology and Environmental Sciences, Biomass and Bioenergy, Industrial Crops and Products, Bioenergy Research and European Journal of Agronomy were accomplished.

The twinning between EU and Argentina was held in Buenos Aires, Argentina, in November 2014. In this event the perennial grasses had been selected as a common topic for presentations and discussion. The event included presentations from both counterparts so that similarities and differences were detected for further discussions. In the framework of the AAIC 2014 international conference that took place in Athens, Greece, OPTIMA project was presented separately as a parallel workshop. In total eight presentations were made from the OPTIMA partners and the progress of the project mainly on agronomic part was presented. It should be pointed out that the conference participants were very actively joined this workshop and a lot of questions had been raised. The majority of the AAIC members are from USA and Latin America and were very interesting with the research that was carried in OPTIMA. A close co-operation between OPTIMA and the two projects – OPTIMISC and Grass Margins – which work on perennial grasses under the call KBBE-2011-5, has been established. The kick off meeting was the first opportunity. The collaboration continued in the organisation of a common workshop in June 2014 in Dublin and a common conference in Stuttgart in September 2015 (see below). From the second year of the project a link was developed with FIBRA project (www.fibrap7.net) that resulting in the organisation of a common summer school in July 2014 in Lisbon. The title of the summer school was “Lignocellulosic crops as feedstock for future biorefineries” (http://www.fibrap7.net/SummerSchoolLisbon.aspx). In the beginning of fourth year of the project a link was established with WATBIO project that ended in the co-organisation of a common conference in September 2015 (in collaboration with OPTIMISC, GRASS MARGINS and FIBRA).

Task 8.2 OPTIMA events

Two important events were originally planned in the OPTIMA. A workshop in month 24 and a conference in month 47 or 48. During the project lifetime a total number of four workshops had been carried out as well as a final conference. The workshops that had been organised were:

- 1st workshop, Join workshop among OPTIMA, EUROBIOREF & FIBRA entitled “Can EU agriculture feed both the energy and bio-based industries of the future in a sustainable way?“ - Parallel event in 21st European Biomass Conference, 6 June 2013, Copenhagen, Denmark.

- 2nd workshop, Join workshop among OPTIMA, OPTIMISC and GRASSMARGINS in the framework of the Federation of European Societies of Plant Biology (FESPB/EPSO) conference 24 June 2014, Dublin, Ireland.

- 3rd workshop, OPTIMA workshop (16/9/14) as parallel event to the The Association for the Advancement of Industrial Crops – AAIC 2014 conference entitled "Industrial Crops and Products“ 14 September 2014, Athens, Greece.

- 4th workshop, OPTIMA workshop entitled “Perennial grasses for biomass production in EU and Argentina”, 11 November 2014, School of Agriculture – University of Buenos Aires, Buenos Aires, Argentina.

The final conference, titled “Perennial Biomass Crops for a Resource Constrained World” (www.biomass2015.eu) took place in Stuttgart from 7 to 10 September 2015. This conference was organised by five research consortia that are funded by the European Union’s Seventh Programme for research, technological development and demonstration. The grant agreements are: GrassMargins, FP7-289461; OPTIMA, FP7-289642, OPTIMISC, FP7- 289159; WATBIO, FP7-311929; FIBRA, FP7-311965. In addition, research reported at the conference includes work from the Biomass for the Future project (BFF), which is funded by the French government. The conference hosted 130 scientists from 26 countries; 107 scientific contributions were presented during the plenary sessions, the nine parallel sessions and the poster session. The final conference covered a wide range of topics presented below:
- Ecosystem service provision and multifunctionality (Perennial biomass crops for development of multifunctionality and maintenance of ecosystem services in agriculture. Energy balances, LCA, carbon sequestration, land use change, use of marginal lands to grow perennial biomass crops, soil erosion, biodiversity effects and other environmental implications of perennial biomass crops);
- Crop selection (The assessment of candidate perennial biomass crop species and their varieties);
- Response to drought stress (Tolerance of perennial biomass crops to drought stress);
- Respose to other abiotic stress (Tolerance of perennial biomass crops to other abiotic stresses: e.g. cold, chilling, flooding, salinity);
- Applications – Example from practices (Examples of practical cultivation and use/applications of perennial biomass crops);
- Conversion and uses (Biomass conversion technologies and different uses of biomass as feedstock and construction materials);
- Biomass quality (Characterization and tailoring of biomass for enhanced conversion into different products; crop management and post-harvest processing);
- Crop management and logistics (Crop production techniques from seed bed preparation to harvest. All aspects of mechanisation for growing, harvesting, processing and logistics of fibre and perennial biomass crops);
- Plant breeding methods and biotechnology (Conventional and next generation breeding methods and strategies. Characterization of genetic resources for breeding and plant biotechnological methods for crop improvement).

Conference content (abstract and presentations) are available at the following link: www.biomass2015.eu. It was given the opportunity to submit full papers to be published in a Springer book or in a special issue at the Global Change Biology Bioenergy (GCBB) journal.

Task 8.3 Website-Intranet (www.optimafp7.eu)

The development of the project’s website constitutes the main communication tool for the efficient dissemination of the project findings. The data gathered and developed from all work packages can be displayed in an effective and user-friendly way. The website encloses all the information that will be available to the public (description of the project, objectives, work packages, consortium, links with relevant issues, events). The website includes a public and a restricted area.

In the public area, the website is structured as follow: (i) Home page, where the concept of OPTIMA is described; (ii) Project, where a window displays objectives, work packages, deliverables and milestones; (iii) Partners, where the list of partners, acronym, address, and contacts is available; (iv) Management to show the hierarchy of OPTIMA; (v) Dissemination, where it is possible to add all the relevant info on publications, workshops, newsletter, congress and handbook; (vi) Meetings, where all the meeting related to OPTIMA, as well as to perennial grasses is available; (vii) Events, where all the relevant events linked to OPTIMA were uploaded.

The restricted area, where an username and password is required, is structured as follow: (i) Partners activity (partners involved in the same activity have the opportunity to share, for example protocols and update its own activity in regard to what planned in the DoW); (ii) Communication (partners can discuss about the problem faced or just communicate that a protocol has been uploaded. There is a hierarchy for sharing info with the Coordinators, WP leader, Task leader and Partner involved in the same or linked activity); (iii) Deliverables (partners can upload the deliverable and then the Coordinator can download and review before to submit it in the ECAS system; (iv) Contacts of the Coordinator, partners and project manager; (v) Support - contact of the OPTIMA webmaster; (vi) Meeting (it is possible to download the agenda, all presentations carried out in every meeting); Documents and Formats (useful document and formats available to every partner for helping them on technical report, financial report, deliverables template, etc); (vii) Logout - to leave the restricted area.

3.9.3 Impact on other Work Packages

Since the dissemination of OPTIMA followed RTD findings (WP1 to 7), the impact generated by WP8 was at scientific and academic level, decision makers, industrial players and stakeholders involved with perennial grasses.

3.9.4 WP8 Deliverables

Overall WP8 results are available on the deliverables (D8.1-D8.9) submitted throughout the 4-year project.

3.10 WP9 Project management

3.10.1 Aim
The overall management plan aimed to achieving the following objectives:
- To coordinate the project between the partners and the European Commission;
- To coordinate the steering committee as well as the group of the WP leaders in order to ensure that all OPTIMA beneficiaries and in particular the WP leaders will carry out the work plan as it has been scheduled;
- To prepare protocols and to ensure that all the fields of the OPTIMA will be carried out according to scheduled work plan;
- To consolidate the OPTIMA results into periodic reports;
- To deliver the financial periodic cost statements to EU;

UNICT was the WP leader with the contribution of the whole consortium.

3.10.2 Project Scientific and Technical Results
During the 4-year project, every partner has actively participated in the management activities of the project, like the preparation of action plan, OPTIMA meetings, workshops and final conference.
Fluent communication has been maintained between the coordinator and project manager, work package leaders, task leaders and partners involved, as well as between every partner.

Following the mid-term evaluation (carried out by two external experts at year 2), detailed discussions concerning research activities of every work package, task and subtask to homogenize experiments were carried out, resulting in join research papers published at year 4 in the frame of the OPTIMA special issue in Bioenergy Research journal (http://www.springer.com/life+sciences/plant+sciences/journal/12155).

List of project meetings, dates and venue
The kick-off meeting was held in Catania, Italy from 07 to 09 February 2012. The kick-off meeting was organized in combination with the “sister projects” GRASSMARGINS and OPTIMISC. It was organized by UNICT.
The first annual OPTIMA meeting was carried out in Perivolos, Santorini, Greece), from October 1st to October 3rd 2012. It was organized by CRES.
The second annual OPTIMA meeting was carried out in Madrid, Spain, from September 30th to October 2nd 2013. It was organized by UPM.
The third OPTIMA meeting took place in Rosario, Argentina from November 11 to November 14 2014. It was organized by INDEAR partner in collaboration with UNICT. In these days a Workshop at the School of Agronomy of Buenos Aires University was also performed.
The fourth annual OPTIMA meeting took place at the University of Hohenheim, Stuttgart (Germany) on 7 September 2015. The room and facilities were kindly provided by Prof. Iris Lewandowski, coordinator of OPTIMISC project, a day before the starting date of the “Perennial Biomass Crops for a Resource Constrained World (from 8 to 10 September 2015)”, the conference organised by the EU FP7 - KBBE-2011-5, KBBE.2011.3.1-02: Perennial grasses: optimising biomass production - SICA funded projects, such as OPTIMISC, OPTIMA, GrassMargins, in co-operation with WATBIO, FIBRA, the French BFF project and the International Miscanthus Society (MEG).
The minutes of each annual meeting have been submitted after one month by the end of the meetings, as scheduled in the DoW.

3.10.3 Impact of possible deviations from the planned milestones and deliverables
- The deliverable “D1.6-7 Report on impact of delayed harvesting, programmed cell death and stress factors” was due in September 2015-16, respectively. Due to the externalization of cation analysis needed for this plant material, this deadline could not be met and UCD proposes January 2016 as the new deadline for this deliverable. The mentioned analysis and some biomass analysis are being carried out at the moment and will be finished by the end of the present year.
- The deliverable “D1.8 Miscanthus and Switchgrass; PhD thesis” was due in September 2015. This deadline was not met due to several factors detailed as follows. Due to medical reasons, the PhD student (Mr. Ángel Cordero) was not able to conduct research from January to February 2014, delaying some biomass analysis and the establishment of the final experiments. This had an impact on the overall timeline of the activity. This impacted, in particular, the final harvesting of plant material and subsequent chemical analyses that are still ongoing. In the last 6 months Professor Osborne’s laboratory (UCD) has also been relocated to a new building and this has disrupted much of the laboratory work. For these reasons the 2015 submission deadlines (three times per year) for PhD thesis in University College Dublin could not be met. The new submission date assigned for this thesis is May 2016. Therefore, UCD proposes June 2016 as a new date for this deliverable.
- Due to the late incoming mass & energy balances (see Task 7.1.2) the work in Task 7.4 was delayed. Moreover, some adaptations and harmonisation of calculations were needed, especially with Tasks 7.2 and 7.3. As a consequence, Deliverable D7.10 (version 1) was finalised in M46. However, no impact were observed for the present and linked activities.
- It was suggested to merge deliverable D8.9 & D8.10 (Handbook with the recommendations for perennial grasses cultivation), leaded by CRES in order to be able to include the most important findings from the growing periods of OPTIMA. A final deliverable was submitted at M48.

3.10.4 WP9 Deliverables
Overall WP9 results are available on the deliverables (D9.1-D9.10) submitted throughout the 4-year project.

Potential Impact:

4. Potential impact and main dissemination activities and exploitation results

Perennial grasses have been widely used as fodder crops for centuries, often contributing significantly to the energy supply, especially at the farm level, but with the advancement of the technological revolution their protagonist role diminished drastically. The limited availability of perennial grasses genotypes adapted to diverse environments and to low input cultivation is a major cause for their scarce cultivation for energy or other bio-based products. In the 21st century, however, perennial grasses may be set to a comeback through a number of different end-uses (e.g. bioenergy, biofuel, biobased products, etc.). However, the opportunity to introduce perennial grasses such as switchgrass, giant reed and miscanthus in southern Europe as multipurpose feedstocks will depend on their economic benefits which are strictly connected with the optimization of agricultural techniques, yield levels, genetic improvements, and qualitative characteristics of the primary products destined to the industry and power plants.

The European Council endorsed a mandatory target of a 20% share of energy from renewable sources in overall Community energy consumption by 2020 and a mandatory 10% minimum target to be achieved by all Member States for the share of biofuels in transport petrol and diesel consumption by 2020. To this end, the OPTIMA project addressed the challenge to develop perennial grasses as bioenergy/bioproduct crop systems, suitable for large scale deployment in Europe in areas unlikely to be used for food agricultural production. It is expected this crops will have a beneficial economic impact on marginal or less favored areas (LFAs) of the Europe Union.

OPTIMA approach aimed at linking the research proposed by including industrial end-users in the project. This should allow the output of this research to develop in a commercial context as rapidly as possible. The major goals of this multidisciplinary network were to evaluate the existing genotypes; to characterize and deliver novel species; to deliver sustainable crop management practices (from sowing/planting to harvest); to evaluate the suitability of perennial crops as a source for biobased products for energy and green materials; to assess the environmental impact through an integrated assessment of sustainability (social, environmental and economic).

Impact of WP1
In general the physiology studies on specific targeted traits would allow developing appropriate breeding programs oriented to the generation of perennial grasses with better characteristic adapted to pedoclimatic conditions prevailing in Mediterranean areas. Thus new insights to tackle crucial bottlenecks for the introduction of perennial biomass grasses in poor and harsh environments were generated. In specific, the information generated in WP1, regarding to the relative growth rate of the grasses at their early stages under different water regimes and saline conditions allowed to identify traits associated with early emergence for better crop establishment. Direct stem transplanting of Miscanthus after stem-bud activation was successfully achieved also in field conditions. The time of stem transplant, influenced shoot density and, hence, biomass yield. An effect of air temperature was observed, with higher minimum temperatures enhancing new shoot emission, suggesting that temperatures close to 20 °C would be ideal for the successful establishment of activated stem cuttings. An important factor to take into consideration is, however, the availability of water. Moreover, the research on mycorrhizal infection improved the resource capture capacity of giant reed and therefore increased plant productivity under establishment phase and adverse conditions that can be of great interest in degraded lands, where soil biota can be scarce. In giant reed, early inoculation with AMF of micropropagated plants can be an effective method to improve the acclimatization process and
Identified traits such as osmotic regulation, photosynthesis and transpiration efficiency under salinity and water stress conditions allowed to screen genotypes with improved performance under water deficits and salinity conditions. The identification of senescence and regulation of metabolite degradation traits helped to identify the cell death process and thereafter decide on ways to induce or delay cell death in senescing leaves directly impacting on the capacity for rapid drying and therefore improving the capacity of the crops of producing more and better biomass of determined quality. The overall performance of M. x giganteus was affected at NaCl concentrations above 60 and 90 mM NaCl. This level of salinity, which equates to 5.4 and 8 dS m-1, proved to be the tipping point where the effect of increasing salinity significantly reduced plant biomass and increased ash content, properties associated with combustion quality. In giant reed (Arundo donax L.) ecotypes we have imposed a high level of stress, which will be rare under field conditions, but this approach was used to physiologically characterize the behavior of giant reed under adverse conditions. Different responses were observed within ecotypes, which would need further studies at molecular levels. In general, lowland genotypes (Alamo and Kanlow) showed a bigger reduction in biomass across different salinity levels, whereas Trailblazer did not show a salinity-dependent reduction in final yields. Delayed harvest could help reduce the water content (such as relative water content) and therefore improve quality of biomass if there are no biomass losses and mechanization is not affected.

Impact of WP 2
At biotechnology level, experienced researchers were engaged in a genomic study of the promising grasses and development of large-scale molecular markers based on it, which provided important information and efficient molecular tools for collecting, characterizing and utilizing these grasses. The molecular markers developed in this proposal would be available to all the consortium partners to be applied to the breeding of plants tolerant to drought and salinity in a later stage (out of the horizon of the present proposal).

A solid base for future QTL detection has been established for switchgrass. The generated raw data and all the variants identified per ecotype totalize about 70 Gb. All these data will be available to all the consortium to be applied to the breeding of plants.

The use of Hiseq 2000 Platform undoubtedly enriched the data volume and the general results. After completing the molecular characterization of all F1 candidate plants by the end of 2015, the greenhouse evaluation of F1 candidates for salt tolerance will be assessed. Field trials will be also completed by April-May 2016. All these results will be available to the Consortium even though out of the horizon of the project with important information on salt tolerance of switchgrass for marginal areas.

Combing with the phenotypic data collected in giant reed, the genes related to drought tolerance will be identified in further. Furthermore, an AD-test can be performed through whole genome-wide to identify the associated loci with drought tolerance. These results will be available to partners to identify ecotype of giant reed suitable to drought conditions of marginal areas.

Impact of WP 3
The agronomic management of perennial grasses is at its infancy stage, compared to the well-known conventional crop, therefore the OPTIMA project, through the adoption, adaptation and development of appropriate agricultural practices for crop establishment, growth, development and harvest, aimed at generate practices that would optimise productivity. Specific bottlenecks for the introduction of perennial grasses at farm level were tackled, like sowing and planting strategies, cultivation under stress conditions and best harvest time for homogeneous feedstock.

Thus, the generated knowledge would allow farmers to diversify their activities, without incurring in costly and risky operations. OPTIMA addressed also the suitability of perennial endemic grasses widespread in Mediterranean area as source of feedstock for bioenergy production and eventually to offer ecosystem services.

Nevertheless, taking into account both the high inter-annual variation of biomass production in Mediterranean rainfed agricultural systems and the high instability of energy markets, it is important to consider not only the biomass production levels of the potential bioenergy species, but also the ecosystem services (erosion control, carbon sequestration, soil fertility), the alternative uses and other direct economic advantages they could offer. It should be emphasized that the number of species currently available for bioenergy use is restricted, while on the contrary in the Mediterranean basin there is a remarkable plant diversity still largely unexplored. Moreover, marginal areas are usually environments with strong limitations
and fragile ecosystem (often classified as protected areas) where plants with excellent adaptation are needed, so it is appropriate to focus on site-specific species with low demand for inputs. Ecosystem services would confer plasticity to the use of these species since their profitability would depend not only on the bioenergy market. In such a context, plant production has to be flexible in order to adapt to both environment and market variability and, also, the ecosystem services provided by small farming agriculture should be taken into account from a socioeconomic point of view. In this sense, native perennial grasses can be considered as multipurpose species because of both the ecosystem services that they provide to the society and their alternative uses.

The research on endemic grasses highlighted that there is a great potential among the Mediterranean flora to provide new species for bioenergy production or ecosystem services, e.g. Saccharum spontaneum spp. aegyptiacum, different accessions of Piptatherum miliaceum. However, further research is needed for getting most of the promising traits of these endemic well adapted species. For example, VAM inoculation at the nursery could be a useful practice to enhance plant performance during the initial developmental stages under field conditions, as found for Oryzopsis miliacea. We believe that the identification of the most appropriate crop type (e.g. drought or flood tolerant, extreme temperature resistant, low resource-requiring, capable of growing on degraded nutrient-poor soils, etc.) to grow on specific unused lands, is the starting point for future breeding programs and may serve as genetic basis for increasing the productivity and stress tolerance of putative bioenergy crops.

The new insight into establishment of switchgrass, miscanthus and giant reed showed that improvements were made. Among abiotic factors to take into account, temperature seems to play a critical role, but only when water does not represent a limiting factor. The proposed hydro-seeding technique of switchgrass underperformed conventional seeding, although results were not negative towards this technique. Interesting opportunities under certain circumstances were observed once the hydro-seeding technique is optimized (i.e. specifically optimized mulch for this species, identification of the best sowing time). In giant reed optimization of factors such best transplanting time (air temperature), node position and stem node pretreatment might strongly enhance rooting rate of stem-cutting propagation technique. In optimal conditions, stem cuttings might be considered as an alternative and feasible technique to increase propagule ratios, decrease establishment costs and decrease environmental impacts associated with rhizome processing, indicating this method as being suitable for nursery activity. Direct stem transplanting of activated stem buds of miscanthus was successfully tested. Temperature close to 20°C is ideal for transplanting, but again only when water does not represent a limiting factor. Among the screened ecotypes of giant reed, accessions of Miscanthus, cultivar of switchgrass it was observed a great genetic diversity worth to be further studied. For instance, drought tolerance of two miscanthus accession, was registered. As compared to Miscanthus x giganteus, an even higher biomass yield was achieved in both irrigated and rainfed plots, suggesting further studies on these accessions in environment characterized by drought conditions, as those of south Mediterranean area. Other accessions, would be worth to be further studied by using mild water conditions due to their extremely high biomass yield in moist conditions. In switchgrass, both lowland and upland, the irrigation rate at 50% ETm increased the yields as compared with rainfed conditions, with higher impact in growing season with lower rainfalls. No differences were found between 50 and 100% ETm suggesting that combining meteorological information and soil moisture measurements, irrigation can be maintained at around 50% of field capacity and optimize resource use efficiency.

When two harvest times were compared (autumn vs winter harvest), winter harvest increased biomass yield and quality, representing a more suitable feedstocks in both thermochemical (moisture and ash content) and biochemical conversions (NDF, ADF and ADL content) for both giant reed and Miscanthus x giganteus. In switchgrass when the harvesting was delayed the moisture content was decreased. Regarding the yields different trends were recorded, suggesting further studies to determine the best harvesting time. The age of the plantation as well as the specific climatic conditions in each growing season could play an important role on the appropriate harvesting time.

Finally, a spatial GIS analysis performed to identify saline and prone-saline agricultural areas available for growing giant reed in Spain might serve as starting point for mapping marginal lands in Europe for perennial and other industrial crops.

Impact of WP 4
Specific bottlenecks for the introduction of perennial grasses at farm level, and to reduced input management were tackled. In this WP, intercropping on marginal lands, productivity in marginal large fields, long-term productivity of exiting large scale plantations, ways to reduce biomass losses during harvesting and storage, organization of crops logistics for the various end-
uses was evaluated. Thus, the generated knowledge would allow farmers to diversify their activities, without incurring in costly and risky operations. Harvesting and subsequent logistic optimization might impact the final biomass yield and calculations. For instance, not recovered biomass during mechanical harvesting was found considerable revealing that there is still great possibility to improve the actual yield through an appropriate set up of baling machines and timely harvesting. Autumn harvesting appears a better option for switchgrass compared to killing frost harvesting for North Mediterranean sites due to winter snow and unfavorable weather conditions that may occur in winter. Nonetheless, it would be important to understand the effect of early harvesting period (autumn) on plant life span as mineral translocation will be interrupted at the likely expense of long term rhizome vigor. On the other hand, in south Mediterranean, winter harvest seems to be the best option to harvest giant reed and miscanthus for both quantity and quality of the biomass. Long term studies can greatly contribute to a more comprehensive view of the real potential of these crops in Europe. Long-term yields of switchgrass, giant reed and miscanthus grown in the Mediterranean basin under low input management (i.e. rainfed and without yearly fertilization) were highly variable across years and locations. Generally, giant reed and switchgrass were the highest and lowest yielding crops, respectively. Compared with switchgrass and miscanthus, giant reed also showed lower yield variability over time and across locations. Such variability may entail practical implications for planning and developing new value chains.

Impact of WP 5
OPTIMA addressed the suitability of the perennial crops as a source for biomass and new-plant derived bioproducts by focusing on the determination of the characteristics for energy and biobased products of the perennial crops, technology assessment of promising pathways for conversion into energy products, pilot tests with secondary conversion technologies for energy and several main biobased materials, like pellets, fibers and paper pulp.

Results on fuel quality confirmed that delayed harvest and nutrient leaching have a positive effect on the characteristics for energy use. It should be recognized that generally herbaceous plants have some advantages over woody ones, which can be referred to biomass yield and stress tolerance, biomass recalcitrance, less energy for comminution and ease cultivation and harvesting. However, disadvantages might be related to the heterogeneity of the feedstocks, which in turn affects bulk density, the ash content, low heating value (LHV), mineral content and ash melting behavior.

Out of the species tested, Miscanthus might be better indicated for thermochemical purposes, owing lowest ash and moisture at harvest, low leaf to stem (mainly M. x giganteus), high LHV and ash melting point; however, the low biomass yield might limit its cultivation in Mediterranean areas without the use of irrigation water as agronomic input. The naturalized species, as Saccharum and giant reed, showed high biomass yield in rainfed conditions, high moisture, low lignin (giant reed) and high hemicellulose content (Saccharum), which might be more suitable for biochemical conversion. However, agronomic and technological strategies may considerably improve the quality of herbaceous biomass; for instance, late harvesting date, field drying, bailing, use of growth regulators, biomass densification and pretreatment are all alternatives to be considered to decrease moisture, ash, mineral content and increase the bulk density, LHV and ash melting temperatures are all recommendations for further research.

Regarding the secondary conversion technologies, torrefaction and pyrolysis are considered as promising pretreatment and conversion technologies for biorefineries and demonstration plants are currently under construction at full commercial scale. However, up till now mainly woody biomass is used as feedstock and only limited data is available for herbaceous crops like Arundo and Miscanthus. For the torrefaction experiments, based on the limited tests, both crops are considered suitable for conversion via torrefaction. In the pyrolysis test the Miscanthus material yielded more liquid than Arundo, with higher energy and carbon yield as well. It is expected that the higher yield of Miscanthus compared to Arundo is also caused by the lower ash content of the Miscanthus feedstock. Typically wood contains less ash and results in high pyrolysis liquids yields or in torrefaction char yields. When the ash content rises, the pyrolysis liquid yields and torrefaction char yields decline. This is in line with the Arundo and Miscanthus tests. Based on budgetary limitations, additional tests are required in the future with a larger number of samples and under varying reaction conditions to draw more general and reliable conclusions.

Perennial grasses might be successfully used in the second generation sugar platform to produce a large number of products,
i.e. fuels and chemicals. However, various factors effecting reducing sugar production during pretreatment method, which has been indicated as a key feature. Possible other uses include animal bedding, insulation materials and packaging materials and biocomposites. New chains needs to secure revenues and therefore must identify immediate markets with a low level of regulations and a simple preparation or conversion process: animal bedding mulching are very convenient market. However, hygiene standards and barriers are often unexpected and will ban raw material from the bedding and mulching markets.

Impact of WP 6

At environmental level OPTIMA explored specific environmental implications of perennial grasses on marginal lands, with particular emphasis on the assessment of CO2 and water exchange carbon storing, energy balance, phytoremediation and soil erosion mitigation at a whole crop level, i.e. measuring the above- and belowground biomass and monitoring continuously the gas fluxes by the newest technologies. Perennial grasses, due to the crop’s low fertilizer (particularly N) and water requirements has an improved energy balance, contaminating and lower GHG emissions compared to most conventional crops such as maize. Perennial grasses, once well established, exploit their potential fully, therefore will be at the top list of the most GHG-saving and less environmental impacting crops. Thus, their cultivation will not lead to environmental degradation, while making efficient use of scarce rainfall and soil water reserves in dry areas. Unlike annual crops, perennial grasses do not render soils vulnerable to erosion because its extensive root system contributes to the build-up of soil organic matter, especially when well established. Where soil erosion is a potential issue, no-till, direct-seeding systems should be considered, which provide for continuous ground cover through the establishment phase.

The information generated on the assessment of CO2 and water exchange between canopy and atmosphere of switchgrass, to our knowledge, the first experience carried out in Europe using an eddy covariance system for four consecutive growing seasons showed that the ecosystem respiration was lower than the C sequestration. However, water availability represents a key factor to photosynthetic activities and to achieve stable and high productions in switchgrass.

The root development and carbon storage in the soil showed that carbon sequestration by the root and rhizome system of perennial grasses is considerable over the stand life time. However, this soil-rhizome accumulation can become prejudicial if land use will be changed, by release of the stored carbon.

Effects on the energy balance due to the use of marginal soils (including contaminated soils with heavy metals) was assessed. The net energy obtained from the production and use of miscanthus grown in marginal soils was dependent on yield level. The lower the yields obtained, the lower the net energy value being obtained. However, the energy balance results needed to be complemented with other parameters, such as the nitrogen emissions or the quality of the biomass for combustion, in order to understand the benefits and constraints associated with growing perennial grasses in marginal soils.

Perennial grasses are able to phytoremediate contaminated soils and to phytotreat waters and wastewaters. Giant reed and M. x giganteus can be considered as interesting candidates for Zn phytoextraction, favored by the metal accumulation observed and the high biomass produced. Giant reed and Miscanthus genotypes showed to be well suited for phytostabilization of heavy metals contamination as these grasses prevented the leaching of heavy metals and groundwater contamination. Moreover, the production of both grasses in the contaminated soils was not affected by heavy metal contamination or was only slightly affected (reduction in productivity of ca 20%). This indicates that the possibility to reduce metals concentration from contaminated soils by means of phytoremediation using these perennials is of great interest and importance to maintain environmental quality standards and to improve soil quality with relative low costs. Still, more studies are necessary to clarify the mechanisms associated with the absorption and translocation of heavy metals in these perennials, its relation with the growing medium and also the interaction of these contaminants with other elements.

Switchgrass was also able to survive/grow in heavy metal contaminated soils in India. The experiment demonstrated that switchgrass perfectly suited the phytostabilization process as it did not allow the heavy metals to translocate into the shoots or aerial biomass.

Miscanthus x giganteus and Arundo donax were also evaluated in red mud flooded soils collected in Hungary (PRIMUS) after an alumina plant collapsed. It was found that miscanthus and giant reed were able to start growing in pots containing red mud. Miscanthus seemed to be better candidate for remediation as this species showed increased tolerance to the tested unfavorable “growth medium”. The observations showed that starting and living capability of Miscanthus and Arundo on red mud would be good enough for use them to grow on and remediate even pure red mud.
Results on soil erosion mitigation by perennial grasses allowed to point out that perennial grasses showed high level of soil losses at the establishment, mainly due to spaced plant density, thereafter soil erosion was significantly reduced, while nutrient leaching and soil organic matter increased as compared with annual cropping systems.

Finally, the environmental impact assessment (EIA), addressing biomass cultivation, but also biomass transportation and biomass processing and use allowed to point that the biogenic system had low erosive potential, reduced disturbance of soil properties, and minimal hydrological impacts. Lower tillage and high biomass production supported biological and landscape diversity. Yet, as a recommendation, site-specific factors should be used to appropriately match the specific crop and location. Producing perennial grasses on marginal Mediterranean land might be feasible and if appropriately managed would lead to limited environmental side-effects.

Bridging perennial grass production with soil phytoremediation represented an in situ possibility for contamination reduction and stabilization, with low costs. Moreover, introduction of a vegetative cover would contribute to the reduction of erosion and lead to organic matter incorporation in the soil, thus restoring soil properties. It would favor biodiversity and occurrence of soil fauna and microfauna, by providing shelter for invertebrates, birds, mammals and higher cover value for wildlife. It would also contribute to the enrichment of landscape values. Even though, yields could be affected by the metal toxicity, energy savings might be reduced or not guaranteed and biomass.

Impact of WP 7
Perennial grasses grown on previously abandoned land in the Mediterranean area provide potentials for climate change mitigation with comparatively low environmental side-effects, especially if several boundary conditions and recommendations are considered. For instance, it has to be ensured that when occupying marginal land (which is not necessarily unused), indirect land use changes are avoided. Abandoned land does not need to be marginal in terms of biophysically inferior properties to entail such benefits, but the achievement of the OPTIMA project is to bring low-quality, previously abandoned land into production by adopting selected crops and agricultural practices. If utilisation options such as efficient stationary energy generation are chosen, benefits can be achieved which are associated with minor other negative environmental impacts. This is a big advantage compared to many other bioenergy pathways, and in some cases even economic profits are attainable.

Economic analysis has shown that financial sustainability of the cultivation of perennial grasses in Europe is related and depends upon EU policies with regard to the security of energy supply and the protection of the environment. In EU today, the cost of delivered biomass from perennial grasses is somewhere between 65 and 75 € per dry ton, which makes it a rather expensive alternative raw material for energy use within the existing framework of energy prices. Fluctuating oil prices and currency exchange rates increase the uncertainty of any economic forecast and raise the risk of economic and financial strategies.

The support given to farmers differs from country to country and at the moment is related to bio-physical characteristics of lands. A more generous support to European farmers is needed to persuade them to cultivate perennial grasses on marginal lands in order to provide feedstock to conversion processes at competitive prices. This will have beneficial environmental effects, especially in electricity generation (which at the moment burns wood pellets from America!).

Disregarding any existing subsidization on the biomass value chains, perennial grass pellets can make a profitable pathway, although the significant investment of pelleting plants is not recovered fast enough to stimulate investors. Home heating with wood pellets is quickly spreading in Europe, because, even at current low oil prices, it works out cheaper. In South Europe (with less annual heating needs) the benefit is smaller and the investment in new equipment is paid back in more than five years, discouraging potential users. Biomass transformation into bio-oil, 2G ethanol, bio-gas, can only be competitive if subsidized. However, the mere fact that we consider comparing the economics of their products directly with fossil alternatives, is indicative of the substantial progress that has been achieved in the last years. A few years ago, such technologies and products were still in the “wish” list. Combined heat and power from perennial grasses is only financially sustainable in situations where capacity utilisation is sufficiently high. This makes district heating in the South of Europe less attractive (not economic unless subsidized), since the heating period is only 3-4 months in most regions. However, CHP is financially attractive in industrial applications, hospitals, hotels, etc. where heat is demanded throughout the year.

Further optimisation of the cultivation of perennial grasses on marginal land is therefore necessary, but also possible, with
promising results. However, the great advantage of perennial crops, resulting from the long plantation lifetimes of 15 years and more, also means that long-term research, development and pilot projects must be carried out and financed. The specific crop characteristics determine specialty applications, in which cultivation itself may serve environmental protection purposes (erosion protection, decontamination, capturing nutrients). Moreover, under certain conditions and given appropriate technological maturity, economical utilisation options are available even without funding, such as co-incineration in existing biomass-fired combined heat and power stations or pellet-fired central heating systems. These should be utilised in pilot projects. Thus, the cultivation and use of perennial grasses on abandoned marginal land can lead to substantial overall gains in sustainability if promoted and managed properly by politics, stakeholders and developers in science as well as in businesses.

All studied perennial crops are considered highly beneficial to the social criteria adopted in OPTIMA (i.e. contribution to rural economy, local embedding and proximity to market) as they are expected to diversify farming activities, provide new opportunity to farmers and the rural economy and facilitate to improved infrastructure for harvesting, storage, transport and logistic.

Impact of WP 8

Throughout the 4-year project, a total of 181 activities (oral presentations and posters at national and international conferences, workshops and symposiums, flyers, interview, etc.) were achieved. Furthermore, 13 between Master and PhD thesis and 22 peer-reviewed publications in relevant scientific journals and 3 book chapters were accomplished.

Important events were organized during the project lifetime. Four workshops (the first as parallel event to the 21st European Biomass Conference, the second as parallel event to the Federation of European Societies of Plant Biology, the third as parallel event to the Association for the Advancement of Industrial Crops and the fourth at the School of Agriculture – University of Buenos Aires, Argentina, jointly with the twinning event between EU and Argentina, where perennial grasses had been selected as a common topic for presentations and further discussions. The final conference, held at the University of Hohenheim, covered a wide range of topics. It hosted 130 scientists from 26 countries; 107 scientific contributions were presented during the plenary sessions, the nine parallel sessions and the poster session.

The organisation of the mentioned events was significantly contributed in the establishment of strong linkages between OPTIMA and other EU research projects that had relevance with OPTIMA. These projects were the two projects funded within the same call, OPTIMISC and GRASSMARGINS, as well as with other additional research projects, namely EUROBIOREF, FIBRA, WATBIO & BIOTRIANGLE. Furthermore, the strong link between OPTIMA and the Association for the Advancement of Industrial Crops (AAIC) on perennial grasses, enlarged the dissemination horizon of OPTIMA.

OPTIMA findings will increase the awareness on perennial grasses for energy and plant derived bioproducts thanks to the wide visibility of the scientific achievements generated in the 4-year project to plant breeders, farmers, scientists, policy makers and consumers. Stakeholders were regularly acquainted (via presentations at local meetings, brochures, emails, local articles) with the developments accomplished within the consortium during the course of the project. The academic partners, all of whom are well known scientists with extensive networks in academic areas, will complement the work to provide high visibility to major breakthroughs through high quality publications, and presentation at key national and international meetings.

List of Websites:

5. Project website and partners contact list
5.1 Website
The OPTIMA website can be found at the following link:

http://www.optimafp7.eu

5.2 Coordination
The project is co-ordinated by the University of Catania, Italy
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**Documents and Publications** [final1-attachment-to-final-report_15-01-16.pdf]

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