AROMATIC GRASSES BASED ENGINEERED ECOSYSTEM FOR RESTORATION OF MARGINAL LANDS AND ENHANCED CARBON SEQUESTRATION

SUMMARY
of
THESIS

SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY

LUCKNOW

प्रज्ञा शील करुणा

ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy
IN
ENVIRONMENTAL SCIENCE

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2022

Around 12 million hectares of land are degraded annually around the world. In India, almost a hundred million hectares of land are unsuitable for agriculture. Land resource is the foundation of primary production for feeding a fast-rising human population and livestock, as well as for sustaining various life forms and nutrients cycling. India pledged to restore 26 million hectares of degraded land by 2030 at COP-14. Total marginal land roughly covers 13.1 Gha globally, with Latin America, Asia, the Caribbean, and Africa accounting for more than half of the total. The potential of marginal land to produce biomass while limiting the diversion of profitable agricultural land from food crop production has received much interest. Unproductive land, wasteland, underutilised land, idle land, abandoned land, or degraded land are all terms that are used interchangeably when referring to marginal land. The term "marginal land" refers to "low-quality land whose production barely pays its cultivation costs." Marginal lands have a low productivity and cause a considerable barrier to agricultural practises. Land degradation has a deleterious impact on the ecosystem services provided by land systems on a global scale. Biotic and abiotic perturbations induce land degradation, besides growth in human population places enormous demands on land resources. Unplanned urbanisation, conventional agricultural techniques, unsustainable uses of natural resources, and climate change are other major contributors to land degradation.

The United Nations Convention to Combat Desertification (UNCCD), at RIO + 20, set a goal of no net land degradation (ELD-Initiative, 2013). The United Nations General Assembly declared 2021– 2030 as the UN decade on ecosystem restoration, with the goal of massively scaling up ecosystem restoration as a proven approach to combat climate change and improve food security, water availability, and biodiversity. Since the last decade, land restoration, bioenergy generation, and farmer financial viability have been top priorities. Due to the sheer amount of marginal/degraded land available in India, soil C sequestration through restoration of marginal land is highly recommended. Different working groups use the following approaches to achieve the goal of restoration of marginal/degraded lands: soil amendments mediated restoration, sustainable agronomic practises, agroforestry approaches, biomass and bioenergy parks, and microbial assisted restoration. Current methods for restoring marginal land are unsustainable and expensive; chemical and biological methods that cannot be implemented without proper technological input; therefore, it becomes critical to investigate alternative methods of restoration of marginal lands.

Perennial grasses have a higher efficacy for soil C-sequestration than other annual crops, and it has been claimed that C4 grasses with high plant diversity boost carbon capture and storage rates on degraded or marginal soils. Restoration of marginal lands by perennial grass (PG) cultivation is a long-term solution for biomass production, carbon sequestration, climate change mitigation, soil stabilization, erosion control, biodiversity protection, and employment generation. These restoration initiatives are critical for meeting the United Nations' Sustainable Development Goals, as well as providing other advantages such as ecosystem services, renewable energy production, and contributions to rural area’s socioeconomic viability. Planting of high-diversity perennial grasses at various levels of species richness for biomass production and soil restoration is receiving a great deal of attention in this context. Numerous long-term researches on diverse cropping systems with low inputs have demonstrated that they are more productive than monocultures with higher inputs. Several researchers have found that biodiversity enriches biomass production and enables ecosystems cope with environmental stress. Because of the increase in complementarity among various species as a result of niche specialisation, biodiversity benefits increase over time.

Polycultures or mixed cultures, for example, have been found to sustain biomass production, carbon sequestration, and nitrogen leaching reduction up to two times higher than monocultures in various studies.

Numerous species of perennial grasses found throughout India can be divided into two groups: underutilized neglected wild species (species that have only local uses such as brooms, huts, and mats and have never been recognized in mainstream Indian agriculture systems) and commercially domesticated perennial aromatic grasses (PAG). The neglected first group of PG can thrive on marginal lands without any external agricultural inputs. Although marginal lands are recommended as a sustainable resource for PG cultivation, their adaptability to different varieties of PG is limited. This study is based on a biodiversity-based field experiment on the second group. Perennial aromatic grasses (PAG) are extensively cultivated around the globe; Because of their unpalatability, these crops are not directly linked to the food chain and thus serve as an alternative to food crops for phytoremediation activity. Planting them instead of food crops in contaminated/marginal soil results in significant financial gains since they are high-value economic crops. In addition to food crops, a wide range of plant species are grown for their secondary metabolites. They are referred to as "aromatic plants" because they are used to produce essential oils for cosmetics and personal care products, etc. Perennial aromatic grasses (PAG) refers to a group of aromatic grasses of the Poaceae/Grass family.

PAG are ideal candidates for production on marginal lands, and it is highly recommended that they should be grown on these lands to avoid competition with food crops. This recent concept of cultivating PAG at various levels of species richness has introduced a new sustainable approach to tackling the challenges related to marginal lands. The fundamental prerequisite for achieving the goal of marginal land restoration, carbon sequestration, biomass, and essential oil production on marginal lands is the selection of suitable PAG species. PAG should be selected for the construction of an aromatic grasses-based engineered ecosystem for the restoration of marginal lands and enhanced carbon sequestration because these aromatic grasses are fast growing, tolerant of marginalised soil, produces high biomass and essential oils, and also has a higher C sequestration capacity. Moreover, planting PAG on degraded land promotes the ecological value of marginal land. Growing diverse groups of PAG on marginal lands has become more useful as a way of accomplishing the objective of a complete bio-economy. However, if chemical fertilizers are used in the pursuit of economic gains, the sustainability of PAG cultivation on marginal soils may be jeopardised. As a result, a study that examines the impact of biofertilizers on PAG productivity on marginal land is required.

A number of studies have found that using plant growth promoting rhizobacteria (PGPR) is a cost-effective and ecologically sustainable strategy to minimize the use of chemical fertilisers. PGPR are well-known for increasing agricultural crop yields, but they also have the ability to tackle a number of environmental issues. PGPR could play an important role in the restoration of marginal lands. By modulating nutritional and hormonal stability and improving resilience to multiple stresses, these PGPR enhance plant growth and production. A number of independent investigations have been carried out to determine the potential of PGPR for bioremediation of contaminated soil and boosting aromatic grass productivity. However, due to their synergistic and multifunctional properties, the application of the PGPR consortium is receiving an increasing scientific consideration in recent investigations. Since marginal land is an underutilised land resource that can be exploited with the help of plants and microbes. In view of the above discussion, a study titled "aromatic grasses-based engineered ecosystem for restoration of marginal lands and enhanced carbon sequestration" was planned. PGPR has also been applied to enhance C sequestration and restoration activities. Very limited studies investigated PGPRs effects on soil characteristics when PAG is grown on marginal land at various species richness levels.

Therefore, as an objective of this study was to investigates how different levels of species richness of perennial aromatic grasses like *Vetiveria zizaniodes* (L.) Nash (Vetiver), *Cymbopogon citratus* (Lemongrass), *Cymbopogon martinii* (Roxb.) Wats., (Palmarosa), and *Cymbopogon winterianus* (Citronella) and PGPR consortium and PGPR consortium of *P. protegens* (RPB1) *B. paramycoides* (RPB2), and *B. paramycoides* (RPB3) on changes in properties of slightly alkaline soil, and on productivity and economical sustainability of the cultivation of these four species of PAG. It was hypothesized in this study that as grass species richness increased, soil fertility and biomass production would increase as well and in PGPR-treated plots, this would be higher than in non-treated plots. In the current investigations, it was also hypothesized that using the PGPR consortium will improve the growth, yield, and economics of these four aromatic grasses. These investigations would lead to development of diverse communities of PAG that can grow on marginal soils with minimum inputs. Under alkaline soil habitats, functional complementarity and differential environmental responses among constituent species may enable them to evolve as a community to be more productive and stable than monocultures.

These ecologically resilient communities are designed to serve as low-input biomass production, C sequestration, and essential oil production on marginal soils by cultivation of PAG. PGPR used in this study were isolated from the rhizospheric soil of healthy PAG such as Vetiver, Lemongrass and Palmarosa that grow naturally in slightly alkaline soils. All isolates were evaluated for plant growth promoting (PGP) attributes such as ammonia production, indole acetic acid production, siderophores, and HCN production, phosphate solubilization, and asymbiotic nitrogen fixing ability using standard procedures. Drought stress and salinity tolerance of isolated PGPR were also assessed. In vitro test of compatibility of selected rhizobacterial isolates has been also done. Three isolates with the highest PGP activity and stress tolerance were chosen for gene sequence analysis based on the results of PGP trait analysis.

The 16S rRNA sequences of these isolated rhizobacteria were analysed at the National Centre for Microbial Resource in Pune, India. NCBI accession numbers MN566090, MN566093, and MN566094 refer to *Pseudomonas* protegens (RPB1), *Bacillus paramycoides* (RPB2) and *B. paramycoides* (RPB3), respectively. Before the field experiment, the pot experiments were conducted for 1 year. In a pot experiment, the efficacy of three PGPR was assessed individually and in all possible combinations in enhancing the growth and productivity of Palmarosa (*C. martinii)*. Both the pot and field experiments have been carried out under subtropical climatic condition of Lucknow, U.P.

Palmarosa was inoculated with PGPR suspensions of single rhizobacteria (RPB1, RPB2, and RPB3) or a consortium of two rhizobacteria (RPB1 + RPB2, RPB1 + RPB3 + RPB2 + RPB3) or a consortium of three rhizobacteria (RPB1 + RPB2 + RPB3) in an equal amount for the pot experiment. Palmarosa that has not been inoculated with PGPR is regarded as a control. PAG were harvested for 3 times in a year at an interval of 4, 8 and 12 months after plantation in the pot as well as in field (harvesting at 4, 8 and 12 months are considered as first harvest, second harvest and third harvest, respectively). In this pot study, plant height, number of slips plant^{-1}, leaf area index, fresh shoot yield, dry shoot yield, essential oil content (%) and oil yield were estimated three times a year after each harvest using standardised procedures. As per the results of the pot study, a consortium of three PGPRs showed significantly higher increase in growth parameters than a non-inoculated control, single rhizobacteria (PGPR), or a consortium of two rhizobacteria. On harvest at 4 months, the consortium of three rhizobacteria showed significant increases in plant height, number of slips, leaf area index, Fresh shoot yield, Dry shoot yield, and oil yield, respectively, of 6%, 16.74%, 25%, 25.8%, 32.79%, and 38.4% in Palmarosa (*C. martinii*), and similar increment patterns were seen for the next two harvests, at 8 months and 12 months. At the 3rd harvest, a consortium of three rhizobacteria inoculated plants had the highest oil yield of 49.87 kg ha^{-1}. Consortium of three rhizobacteria (PGPR) was selected for field experiment because of its high efficacy compared to consortium of two rhizobacteria and single rhizobacteria during pot study. Field experiment was conducted for two consecutive years (2017-18 and 2018-19) on slightly alkaline soils in the premises of BBAU, Lucknow, U.P.; PAG namely Vetiver (*Vetiveria zizanioides*), Lemongrass (*Cymbopogon citratus*), Palmarosa (*C. martinii*) and Citronella (*C. winterianus*) were planted in all possible combinations at one, two, three, and four species richness levels in a randomised block design (RBD) with three replications and two treatments i.e. with the inoculation of PGPR (hereafter PGPR^{+}) and without inoculation of PGPR (hereafter PGPR^{-}).Species and richness levels specific changes in soil physicochemical parameters (electrical conductivity; EC, bulk density; BD, total soil organic carbon; TOC, and available nitrogen; N_{av}), microbial (colony forming unit; CFU, microbial biomass carbon; MBC) and enzymatic (alkaline phosphatase; ALKP and dehydrogenase; DHA) activities were observed.

The potential of soil C sequestration and biomass C sequestration at different species richness levels in PGPR treated (PGPR consortium) and PGPR untreated plots were also assessed. The changes in growth (height, number of slips and leaf area index), productivity (fresh shoot-root biomass and oil yield), quality (important secondary metabolites) and economics (Cost of cultivation, gross and net return) in response to PGPR were observed at three harvests/ yr for two years. The highest reduction in soil pH, EC as well as increase in soil TOC, CFU, N_{av}, MBC, and enzymes activities, were found at two species richness level of Vetiver and Lemongrass in both $PGPR^+$ and $PGPR^–$ plots followed by monoculture of Vetiver at the end of the experiment. Monoculture of Vetiver produced the highest annual total dry biomass (22.8 and 27.5 Mg ha^{-1} in $PGPR^–$ and $PGPR^+$ plots, respectively), with similar values at two species richness level of Vetiver and Lemongrass in $PGPR^–$ and $PGPR^+$ plots. The highest biomass C sequestration (plant carbon) was found in Vetiver monoculture and two species richness level of Vetiver and Lemongrass. The soil C sequestration potential was highest at two species richness level of Vetiver-Lemongrass and Lemongrass-Palmarosa as well. On an average the highest increase in growth and productivity with the effect of PGPR was found in Vetiver followed by other grasses. This research revealed that using the PGPR consortium to increase the productivity of aromatic grasses on marginal lands can be a cost-effective and ecologically sustainable method.

From the findings of the study, entitled 'Aromatic grasses based engineered ecosystem for restoration of marginal lands and enhanced carbon sequestration' it was concluded that amendment of PGPR consortium increased soil fertility in all species richness levels of PAG over the non PGPR amended plots. However, the maximum microbial biomass carbon was recorded in two species richness levels of Vetiver-Lemongrass (VL) and Lemongrass-Palmarosa (LP) and further increase in species richness level in field caused low microbial biomass carbon. Increase in soil fertility with increasing species richness levels and improvement in soil fertility was higher in PGPR treated plots. Increase in fertility and productivity was not beyond the species richness level two and even not in all the combinations. It seems that soil carbon sequestration potential of marginal land proportional to root and shoot biomass production of the plants. This study reveals that biomass production and carbon sequestration are related with the plant size and not with in species richness level as three and four species combinations could not support higher economic and ecosystem services-based gains in cultivation of perennial aromatic grasses. The production of aromatic essential oils and their net return indicate that the marginal land which generally considered as non-productive for agriculture has a strong potential of C-sequestration potential and economic gain. Monoculture of Vetiver can give highest return as 2094.95 USD (157,196.67 rupees) which cannot be obtained from routine agriculture. Lemongrass, Palmarosa, Citronella can also give good returns in multiple years due to its multi harvesting nature and low input expenditure.