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Biodiversity Enhancement for Improving the Sustainability of Broccoli (*Brassica oleracea* vr. *italica* Plenk) Organic Seed Production

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Abstract: The plant heritage of the Mediterranean basin, with its wide biodiversity, offers the best conditions to pursue the objectives of the EU Green Deal, and among it, we pointed our attention to the *Brassica oleracea* crops, thanks to the high number of landraces and of its wild relative species widespread both in agro and ecosystems. In the framework of the European project BRESOV H2020 "Breeding for Resilient, Efficient and Sustainable Organic Vegetable Production", we evaluated different organic growing conditions and plant characteristics to pursue a good yield and high quality of organic seed under different nutrition protocols. We took in attention the two landraces of sprouting broccoli (*Brassica oleracea* var. *italica* Plenk) Sparaceddu and Cavolo Riccio di Messina, which well express the traditional trait of broccoli Sicilian biodiversity that is often unfortunately underestimated. The results showed that the new tools for organic plant nutrition implement the plant characteristics and the related seed yield also by using a lower level of inputs, achieving economically sustainable production by the use of the nutrition protocols evaluated. There is, therefore, a strong need to affirm the concept of "minimum dose" in order to obtain a satisfactory result in terms of production and quality. The research also focused on the characteristics of brassicas crops, highlighting the main factors that render the use of biodiversity possible and profitable.

Keywords: vegetables; landraces; brassicas; amminoacids; microorganisms; seed yield

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1. Introduction

Seed production plays an important role along the process of agro-biodiversity conservation because it guarantees the possibility for establishing the traditional vegetable supply chains [1]. Its importance is increased in the case of horticulture because at the spatial level, biodiversified products can be represented by landraces and crop wild relatives (CWRs) species of modest extent, able to activate seasonal supply chains that are very limited in time and space, or by CWRs with investments of higher extent [2,3].

In particular, the small-scale horticultural landraces represent an extremely complex and varied production reality, characterized by customs, traditions and habits that cannot be ignored in order to identify correct and effective protection policies. The small-scale approach is fundamental for the sustainability of economies, the organoleptic, nutritional and nutraceutical traits of the products, and could develop its investments and make a profit in both rural and urban environments for each territory [4]. In these contexts, small-scale horticultural production plays an important role for the improvement of food security, both in quantitative and qualitative terms, and for the production of numerous "eco-system services" and social, scientific, educational, cultural, and even aesthetic benefits, despite the presence of a limited market for genetic resources [5,6].

It is well-known that in the case of biodiversity, the seed market can be formal or informal [7,8]. In the first case, horticulturist participation in the seed market takes place and the literature reports that transaction costs are not a barrier to achieving higher levels of intra-crop and unit production yields, showing that these types of markets do not threaten biodiversity [9]. In the second case, seed is often held under monopoly conditions and reproduced with traditional schemes passed down from father to son, which can not guarantee a inconsiderable economic privilege [10,11]. In this latter case, seed market aversion can be traced back to the risk of value loss as a consequence of the commercial use of biodiversity seeds [12,13]. Indeed, cases are known where the horticulture industry obtains samples of landraces/ecotypes conserved by in situ and/or ex situ collections (government agencies, universities and agricultural research institutes, etc.), to derive sources of new breeding lines and advanced elite materials for the industry, demonstrating how horticultural companies are often not fully aware of the implications of the Biodiversity Convention in terms of access and benefit-sharing [14,15]. However, the literature shows how the active involvement of farmers in research and development activities can, on the one hand, create space for social learning and innovation and, on the other hand, contribute to the creation of a dynamic system for the community biodiversity management and sustainable use [16–19].

Closely linked to peasant or small-scale agriculture, such as horticultural biodiversity, is organic farming and the corresponding seed production, because of its useful implications for a complete ecological transition based on agroecology, circular economy and short supply chain, as advocated by the "Green Deal EU", "From Farm to Fork EU" and "Biodiversity EU" strategies [20–22]. Currently, and thanks to the derogation regimes provided in the EU organic regulation, local varieties and self-produced on-farm seeds have been an alternative source of seeds to meet the demand vs. supply balance [23]. At the regional level, however, the protection of genetic diversity through the use of local varieties and landraces is often hindered by the regulations on organic seeds; in fact, according to the provisions in force in the RDPs (Rural Development Plan), access to the premium for "organic agriculture and animal husbandry" is subject to compliance with the requirements outlined in EEC Regulation 2092/91 (art. 6 and 6a) and subsequent ones, concerning the production rules on propagation material. These constraints appear to be extremely binding for those who choose to produce landraces, and a considerable number of potential 'custodian farmers' prefer to abandon their cultivation in order to receive EU aid [24].

Great expectation is placed in the Implementing Regulations of EU Reg. 848/2018, whose implementation has been postponed in 2022 by EU Reg. 1693/2020, for the future mechanisms for granting derogations for non-organic seeds contained in mixtures.

Within this framework, a survey was conducted on biodiversity broccoli (*Brassica oleracea* L. var. *italica* Plenck), considered among the main vegetable foods on the markets due to their high nutritional value, linked to high levels of phytochemicals that have beneficial effects against cancer and other diseases [25,26].

Sicily, at the center of the Mediterranean, plays a major role in broccoli production [27,28]. The conservation of the wide biodiversity of broccoli in Sicily is subject exclusively to the production choices of the few farmers who have a monopoly on their seeds. Currently, there are no common standards governing the propagation and conservation system; the reproduction of the landraces, and of their genetic purity. The plants of the two Sicilian landraces of broccoli are self-incompatible so the cross-pollination could affect the genetic behaviours of the landraces, so the regeneration of seeds in purity is guaranteed by the adoption of specific traditional schemes for conservative selection handed down from father to son (precautions regarding distances, sowing methods, use of nets for avoiding pollination by insects, mass selection in the field for agronomic traits) [29]. Indeed, the preservation of vegetable genetic heritage represents an important market positioning opportunity for these custodian farmers [30]. By satisfying a purely local and seasonal demand and by placing a limited amount of product on the market, these farmers actually enjoy a considerable economic privilege.

At present, therefore, the conservation of many Sicilian vegetable landraces is based exclusively on economic advantage, which may disappear over time. It is therefore essential to set up appropriate conservation policies aimed not only for conservation but also, where possible, for a controlled expansion of local production.

From the above, the problematic picture that characterises the biodiversity of the vegetable production and of the related seed production in Sicily emerges: there is still a lack of an integrated strategy for biodiversity conservation that provides protocols, expertise, and infrastructure for in situ (crop wild relatives), on farm, and ex situ (landraces) conservation according to the principles of sustainable conservation, equitable use and sharing of resources, as required by current international agreements [31]. Biodiversity is often linked to the concepts of resilience, cultural linkage, and territory, but knowledge of the value of the multiple functions performed by safeguarding Sicily's horticultural biodiversity is not always sufficiently clear [32].

The research question was twofold:

- From a technical point of view, to verify what are the optimal growing conditions needed to pursue good yields and high-quality organic seed with the use of new organic low-input tools for plant nutrition;
- From the socio-economic point of view, to identify the factors that condition entrepreneurial behaviour according to the production choice linked to biodiversity, the characteristics and the market for the supply of seeds and the placement of the final product.

The survey was carried out in an area that is particularly suited to the cultivation of brassicas that are the fruit of biodiversity, i.e., Sicily, an area in which conservation strategies have been activated for various *B. oleracea* crops both in their places of origin (in situ or on farm) and in special genebanks (ex situ), such as the conservation centre of the University of Catania [27,28].

2. Materials and Methods

2.1. General Organization of Research

The research was carried out in the broader context of the European H2020 project BRESOV on *Brassica oleracea* crops and allowed, on the one hand, to establish the optimal growing conditions necessary to pursue a good yield and high-quality organic seed using new tools for plant nutrition with low inputs and, on the other hand, to analyze the productive characteristics of vegetable biodiversity in order to identify the socio-economic aspects influencing the preservation of the sprouting broccoli landraces considered.

The main points of the research are shown in Figure 1.

Overall, the research was carried out in two stages. In a first step, a territorial survey was carried out in Sicily to identify the main biodiversified broccoli production areas. The structural characteristics of the representative production units were then identified by means of a questionnaire-form. This phase is important because it not only clarifies the basic reference framework, but also because, as specified below, it allows us to answer the research question on the factors that push entrepreneurs to invest in biodiversity. In the second step, however, once the areas were identified, the availability within a representative company in the province of Catania was asked to activate a split-plot experimental design to verify the concrete possibility to produce seeds of broccoli landraces by low-input organic plant nutrition. This phase is important for facilitating the political challenge undertaken by the EU: the support of biodiversity (EU Strategy for Biodiversity) as the engine of a more sustainable agriculture and in line with the Millennium Sustainable Development Goals (2030) and the EU Green Deal Strategy.

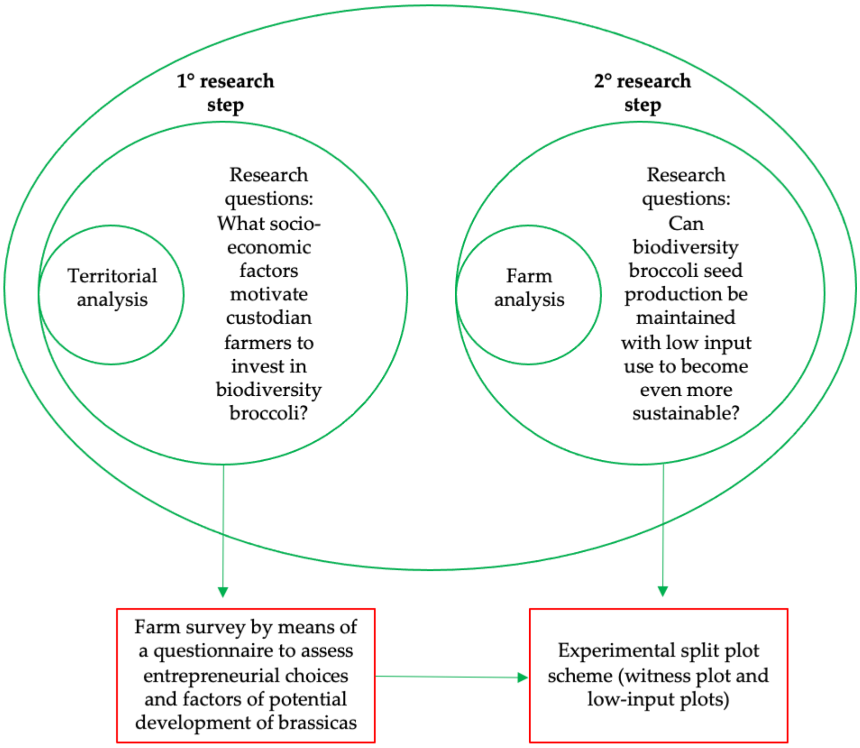

Figure 1. Complementary phases through which research in low-input Brassica seed production was articulated.

2.2. Socio-Economic Analysis of Horticultural Biodiversity

This part of the research was carried out in eastern Sicily on vegetable farms centered on the production of the *Brassica oleracea* biodiversity, run by custodian farmers with specific expertise in the mass selection of local ecotypes.

The surveys were carried out in autumn 2020 using a specially prepared questionnaire form. The variables collected and considered for the survey have been summarized in Table 1.

Table 1. List of variables considered for the analysis of the sample of vegetable farms with biodiversity cultivation in Sicily (*).

Variable	Acronym	Measuring Level
Type of regime	REGIME	Certified organic = 1; Integrated farming = 2; Conventional with good agricultural practices = 3; Conventional = 4
Age	AGE	Under 35 years = 1; Between 35 to 60 = 2; Over 60 years = 3
Accessibility to the informal seed market	SEED_MARK	yes = 1; no = 0
Implementation of agricultural practices to promote the biodiversity of the cultivation environment	BIOD_ENVIR	yes = 1; no = 0
Types of activity	BIOD_ENVIR_ACT	Composting = 1; Green manure = 2; Feeding troughs for wild animals = 3; Bird houses = 4; Hedge maintenance = 5; Wildflower maintenance and pollinator development = 6; Dry stone walls = 7; Care of damaged trees = 8; Wide mesh netting for wildlife mobility = 9

Table 1. *Cont.*

Variable	Acronym	Measuring Level
Using biodiversity to attract customers/consumers	ATTRAC_CUST_CONS	yes = 1; no = 0
Importance of biodiversity for attracting customers/consumers	MARKET	From 1 = low to 5 = high
Using natural farm features to promote the biodiversity market	NAT_ASPECT	yes = 1; no = 0
Importance of natural business aspects	NAT_ASPECT_MARKET	From 1 = low to 5 = high
Biodiversity supply chain	SUPPLY_CHAIN	yes = 1; no = 0
Farm location in Natura 2000 Network Site (SIC/ZPS)	NATURA_2000	yes = 1; no = 0
Role of sustainability in the territory	SUST_TERR	From 1 = low to 5 = high
Influence on landscape	LANDSCAPE	From 1 = low to 5 = high
Pollution of conventional agriculture	POLLUT_CONV	From 1 = low to 5 = high
Ecosystem improvement with organic farming	ECOSYS_ORGANIC	From 1 = low to 5 = high
Crop profitability	PROFIT	From 1 = low to 5 = high
Hydrogeological system influence	HYDROG_SYST	From 1 = low to 5 = high
Ability to maintain biodiversity	BIODIVERS_ABILIT	From 1 = low to 5 = high
Productive address	PROD_ADDRESS	Specialised horticulture = 1; Mixed horticulture = 2; Horticulture and fruit growing = 3
Cultivated Biodiversity Brassica	BRASSIC_CROP	Brassica oleracea var. italica "Sparacello" = 1; Brassica oleracea var. acephala "Riccio" = 2; Brassica oleracea var. italica "Natalino" = 3

(*) Our elaborations.

A total of 8 farms were surveyed, which were considered representative and sufficient in number to represent the peculiarities of biodiversity Brassicaceae.

The data collected were analysed using a classical multiple regression model with p independent variables:

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \cdots + \beta_p X_{pi} + \varepsilon_i \quad (1)$$

where:

β_0 = intercept;
β_1 = inclination of Y with respect to the variable X_1, holding constant the variables X_2, X_3, . . . , X_p;
β_p = inclination of Y with respect to the variable X_p holding constant the variables X_2, X_3, . . . , $X_{p\text{-}1}$;
ε_i = at observation i.

The data were processed with SPSS 26.0 software; the selection of predictors was carried out with a "block" procedure considering, therefore, all independent variables and the *t*-test for predictor goodness-of-fit.

Ultimately, the regression model made it possible to arrive at an articulated answer to the research question of what factors condition entrepreneurial behaviour and, in particular:

1. Is there, based on the independent variables found, a good model able to explain entrepreneurial behaviour in the choice of broccoli biodiversity?

2. What is the importance of territorial location, market aspects, production orientation, cultivation practices inspired by sustainability and access to the seed market in predicting the use of landraces of broccoli?
3. How do sensitivity to sustainability, openness to the market and the ability to build a supply chain influence the quality of broccoli seed self-produced by growers?

Interested firms were informed about the objectives of the research and granted permission to use the data in aggregate (and not individually) for research purposes only.

2.3. Low Input and High Quality Organic Seed Production

Two Sicilian broccoli landraces, named "Sparaceddu" (BR357- UNICT4963) and "Riccio di Messina" (BR358-UNICT5077) were used to carry out the trial (Figure 2). The experiment was conducted in an experimental field of a private organic certified farm in an area called Agnelleria in Belpasso -CT- (37°31′31.5″ N 14°55′04.7″ E). The soil was prepared by tiling it with the use of a disc harrow. Thereafter it was fertilized with 5000 Kg/Ha of an organic pelleted compound called Archimede ©, using the protocol as suggested by Itaka Solution company. Pieris brassicae pest control was performed by spraying the plants with Bacillus turingensis; fertigation operations were scheduled every 15 days using the above-cited nutrition protocols (Table 2).

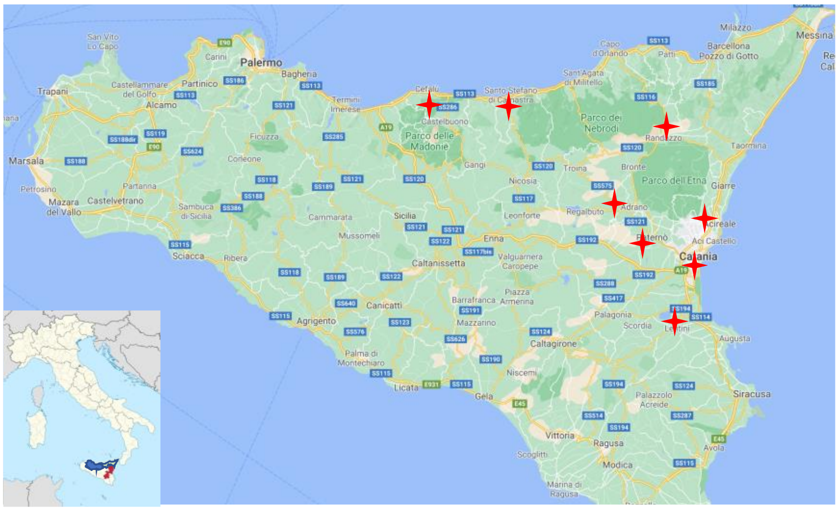

Figure 2. Locations of biodiversity vegetable farms surveyed in Sicily.

Table 2. Nutrition protocol used in the broccoli experiment.

		Nutrition Protocols		
Thesis	**Product**	**Doses (Kg/Ha)**	**N Supplied/Ha**	**Notes**
NP0	None	None	None	None
	3KO ©	5	–	Every two weeks
	Amminocomplex extra ©	80	6.4 kg	Every two weeks
	3KO ©	10	–	Every two weeks
	Amminocomplex extra ©	160	12.8 kg	Every two weeks

The plants were transplanted at the end of November 2019 following an experimental split plot scheme, characterized by three replications with seven plants and an investment of four plants per m^2 [31–34]. Fertilization was done with "pellet manure" with N, 4%; P2O5, 3.5%; and K2O, 3%. The experimental design adopted was split-plot with the main plot represented by the Itaka plant nutrition protocols (**NP**) based on Amino complex© and 3KO© (*Trichoderma harzianum*, *T. asperellum*, *T. atroviride*) products (**NP0** = 0 g L^{-1};

NP1 = 1.5 g L^{-1}; **NP2** = 3.0 g L^{-1}) and the sub-plot by genotype, and each thesis was represented by three replicates of fourteen plants each. Plants were placed at a crop density of 4 plants m^{-2}, along single rows spaced 1 m from each other.

We analysed the response and the growth rate of the different cultivars, and to assess the quality of the organic seeds production, the germination tests and the analysis of the seed yield components were carried out.

After 158 days from transplanting, morpho-biometric tests were carried out on the plants to evaluate the quality aspects and growth response, allowing the collection of important data such as: plant height, weight, stem basal steam diameters, fresh weight, and finally the dry matter produced by each plant.

The surveys on seed production are repeated in 2 years, 2020 and 2021.

The single measure can be schematized in this way:

$$x_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where α_i is a constant factor, linked for example to the treatment and ε_{ij} is the variable that represents the random variability of the data, not controllable and not related to the treatment. The hypothesis is that the variability is of the same entity in the different groups, and that the ε_{ij} are uncorrelated with each other. We will also assume that ε_{ij} is a Gaussian variable with zero mean value and variance δ^2.

3. Results

3.1. Characteristics of the Farms Surveyed

The farms surveyed fall within the peri-urban and urban areas of the metropolitan cities of Catania, Messina, and Palermo. In particular, the municipalities concerned are Adrano, Paternò, Catania, and Acireale in the province of Catania; Roccella Valdemone and Santo Stefano di Camastra in the province of Messina; and Isnello and Palermo in the province of Palermo, typical areas of intensive and specialised vegetable production (Figure 2).

The farms present a prevalent size between 3 and 10 hectares (50%) and are led by young entrepreneurs (25% under 35 years old) or between 35 and 60 years old (62.5%), attesting to an intergenerational interest in the management of the farm (Table 3). In fact, the generational change in these business activities, characterized by a high value of the family social capital, is a widespread custom.

The prevailing productive direction is the specialized vegetable production (50% of the cases), with a high sensibility towards the theme of the productive sustainability (only 12.5% leads the farm in a conventional regime), as the case of activities in organic (25%), integrated (37.5%), integrated and conventional (37.5%) farming with the adoption of good agricultural practices (25%), often as a consequence of "cross compliance system" and "greening" measures incorporated in the CAP or the location of production units within Natura 2000 protected areas or SCI/SPA sites (in 50% of cases positive).

Within the farms surveyed it is possible to find a wide use of vegetable landraces and, in particular, of the sprouting broccoli landraces, with a prevalence of 'Sparacello' (37.5%) and Riccio di Messina (37.5%) ones (Figure 3).

Within the farms it is possible to find a wide and articulated panorama of cultivation practices "friendly" to the environment and biodiversity. Among these, feeding troughs for wild animals (22%), composting and care of damaged trees (about 17% for each item), wildflower maintenance and pollinator development (11%), etc., are the most popular. The wide use of these activities shows how vegetable growers have developed over time a relationship with the territory on which they insist and with all the biotic components present, ensuring its survival.

In the case of the landraces studied, however, it emerged that there are almost no links between the seed companies and the farms, and that almost all the propagation material comes from 'custodian farmers', who are the holders of the biodiversity of these species and of the enhancement of biological diversity. In 75% of cases, in fact, the seed is self-produced.

Table 3. General characteristics of the biodiversity of brassicas farms (*).

Variables	Value	Variables	Value
Utilised Agricultural Area (UAA), %		Age of the entrepreneur, %	
Up to 3 hectares	37.5	Under 35 years	25.0
From 3 ha to 10 ha	50.0	Between 35 to 60 years	62.5
Over 10 hectares	12.5	Over 60 years	12.5
Cultivated Biodiversity Brassica, %		Cultivation practices in favour of the environment and biodiversity, %	
Brassica oleracea var. italica "Sparacello"	37.5	Composting	16.7
Brassica oleracea var. acephala "Riccio"	37.5	Green manure	5.6
Brassica oleracea var. italica "Natalino"	25.0	Feeding troughs for wild animals	22.2
		Bird houses	11.1
Productive address, %		Hedge maintenance	5.6
Specialised horticulture	50.0	Wildflower maintenance and pollinator development	11.1
Mixed horticulture	37.5	Dry stone walls	5.6
Horticulture and fruit growing	12.5	Care of damaged trees	16.7
		Wide mesh netting for wildlife mobility	5.6
Regime, %			
Certified organic	25.0	Farm location in Natura 2000 Network Site (SIC/ZPS), %	
Integrated farming	37.5	Yes	50.0
Conventional with good agricultural practices	25.0	No	50.0
Conventional	12.5		
		Market accessibility of biodiversity seeds, %	
		Self-production	75.0
		Informal market	25.0

(*) Our elaborations.

(**a**) (**b**)

Figure 3. Sprouting broccoli landraces E "Sparaceddu" (**a**) and G "Riccio di Messina" (**b**) found in vegetable farms surveyed in Sicily.

In most cases, these entrepreneurs are 'self-employed' in the preservation and reproduction of genetic material, trying to maintain the intrinsic characteristics that allow respect for the traditional nature of the species, handing down knowledge and skills from generation to generation, avoiding inter-varietal and genetic 'pollution' favored by cross-pollination with other *B. oleracea* crops and their new F1 hybrids. The genetic profile of these landraces, which are populations in equilibrium, could be maintained through the adoption of specific precautions regarding the distances among the other *B. oleracea* crops, the sowing date and methods, the mass selection for agronomic traits, such as for resistances to biotic and abiotic stresses, and for organoleptic traits. Seeds are generally produced through the use of mother plants, used as seed-bearers, selected and isolated, within the cultivated fields by nets. In some cases, the producers supply the seed to nurseries in the area that take care of seed propagation; the nurserymen supply the producers with the required propagation material (seed or seedlings) according to the producer's requests.

3.2. Factors Affecting Seed Production of Quality Biodiversity

In order to understand which socio-economic factors, have the greatest influence on seed production and the preservation of the biodiversity of broccoli landraces, a regression analysis was carried out by combining the area under cultivation (UAA), as the dependent variable, with all the other variables shown in Table 3, as independents or predictors.

The questions that were attempted to be answered were as follows:

- Is there, based on the independent variables, a good model capable of explaining the diversity of behaviours existing between the different realities surveyed?
- What importance do the selected variables have on the choice of the broccoli biodiversity to be cultivated and on the related seed market?
- How does the use of mass selection guarantee the opportunity to multiply the seed and maintain the patrimonial value of biodiversity?

The model constructed in this way explains 61% of the overall variability in UAA between farms, as shown in Table 4.

Table 4. Main results of regression analysis in vegetable farms with seed production of the Brassicas Biodiversity (*).

Summary of the Model							
					Variation of Adaptation		
Model	R-statistics	R-square	Corrected R-square	Standard deviation Error of prediction	Variation of F	Significance Change in F	Durbin-Watson
1	0.717 [a]	0.614	0.534	0.697	6.357	0.045	2.600
			ANOVA [a]				
Model	Sum of squares	df	Mean of squares	F	Sig.		
Regression	48.889	7	6.984	.	0.0000 [b]		
Residue	0.000	82	0.000				
Total	48.889	89					
Coefficients							
Model	Coefficients	Standard error	T-Stat	Significance value			
(Constant)	0.407	0.000	27,852,210.1	0.000			
CROP	0.203	0.000	58,788,023.199	0.000			
PROFIT	0.441	0.000	75,379,260.543	0.000			
BIOD_ENVIR_ACT	−0.008	0.000	−5,180,378.917	0.000			
HYDROG_SYST	0.975	0.000	104,030,143.276	0.000			
NAT_ASPECT	−1.11	0.000	−165,986,518.483	0.000			
PROD_ADDRESS	0.127	0.000	35,806,966.104	0.000			
MARKET	0.169	0.000	64,933,983.30	0.000			
SEED_MARK	−0.737	0.000	−116,750,453.488	0.000			

(*) Our elaboration.

[a]. Dependent variable: UAA. [b]. Predictors: CROP, PROFIT, BIOD_ENVIR_ACT, HYDROG_SYST, NAT_ASPECT, PROD_ADDRESS, SEED_MARK.

Not only that, but the fraction of variance explained is statistically significant. The procedure, after repeated iterations, selected the predictor variables until the importance of what is summarized in the table was given. Therefore, in the choice of the area to be invested in biodiversity, the specific crop or "CROP" (type of broccoli cultivated) is important, since it guarantees a significant recognition to the farm profit and the presence of semi-natural elements in the farm such as "hedges and bushes", "sloping surfaces", "terraces and dry stone walls", which suggest the enhancement of the different realities as agricultural areas with high natural value. These elements can be of direct benefit to farms, especially if associated with good practices such as green manure, crop rotation, grassing, use of compost, and mulching with crop residues, as it also appears in the literature [35,36].

Biodiversity conservation, while providing net benefits to the environment and society (ecosystem services such as food, drinking water, climate regulation, erosion control, and collective well-being), implies costs that cannot be left to farmers alone, but need to be covered by appropriate external incentives. In this respect, both support through funding (public and private) and the diffusion of the biodiversity markets where consumers are willing to pay a premium price for sustainable, healthy, and high-quality food products are of great help. This is demonstrated by the importance attached to the "PROFIT" factor.

The management of the local hydro-geological system "HYDROG_SYST" is a highly significant factor for the preservation of biodiversity and quality seed production, which is why it was chosen to carry out a test on the sustainability of seed production at different input levels. This factor is closely linked to the level of profit and the nature conservation aspects adopted in farm management.

Eliminating "outliers" from the analysis would result in a significant increase in the proportion of variance explained and a "better" distribution of residues, as shown in Figures 4 and 5.

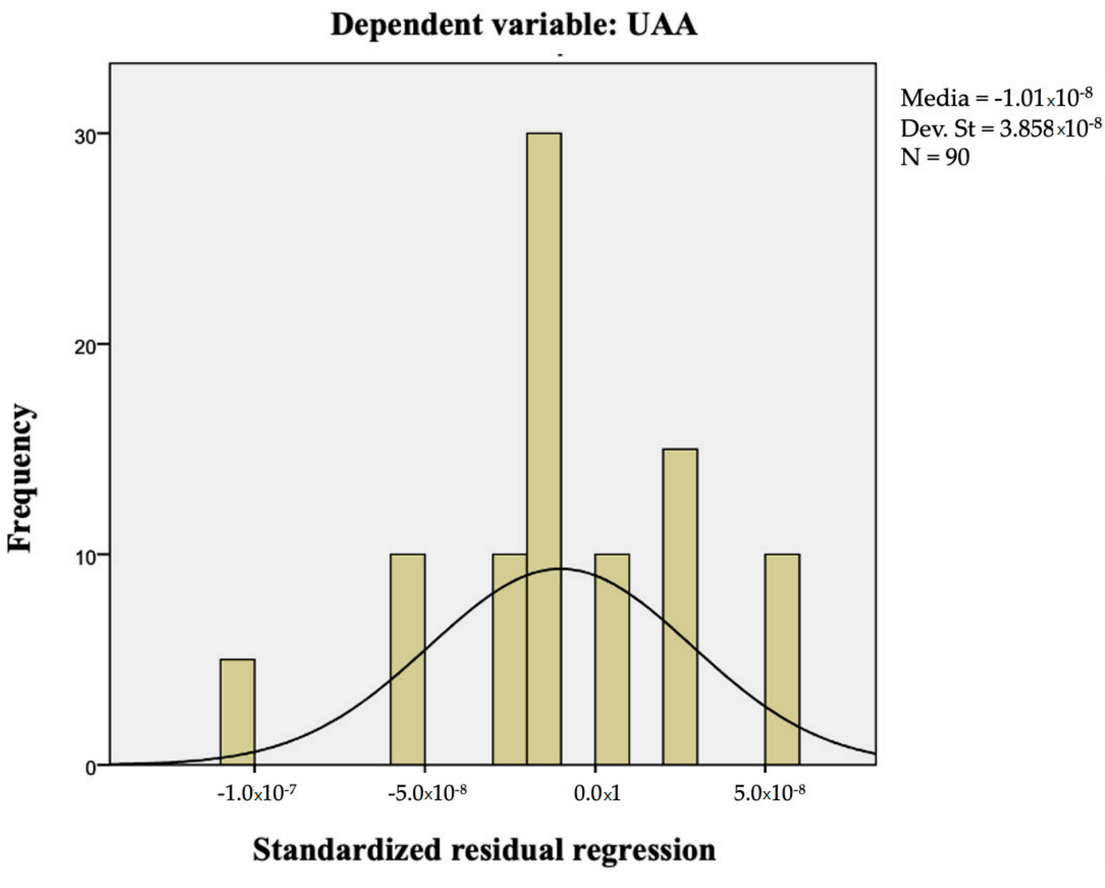

Figure 4. Representation of standardized residues in the biodiversity brassica production model.

Finally, the preservation of the vegetable genetic heritage represents, for the "custodian" producer an important opportunity for market positioning, which aims to satisfy a purely local and seasonal demand, strongly interested in local products that respond to local food customs; In this way, all possible actions to defend and safeguard the aforementioned "patrimonial value" are activated (consisting not only of the actual seed but also of the system of knowledge that is sedimented on-site and handed down from generation to generation, which allows, through mass selection, the identification of plants capable of maintaining the characteristics of the vegetable unaltered, organized, and managed by aggregations of farming families ("SEED_MARK"), as it also appears in the literature [37,38].

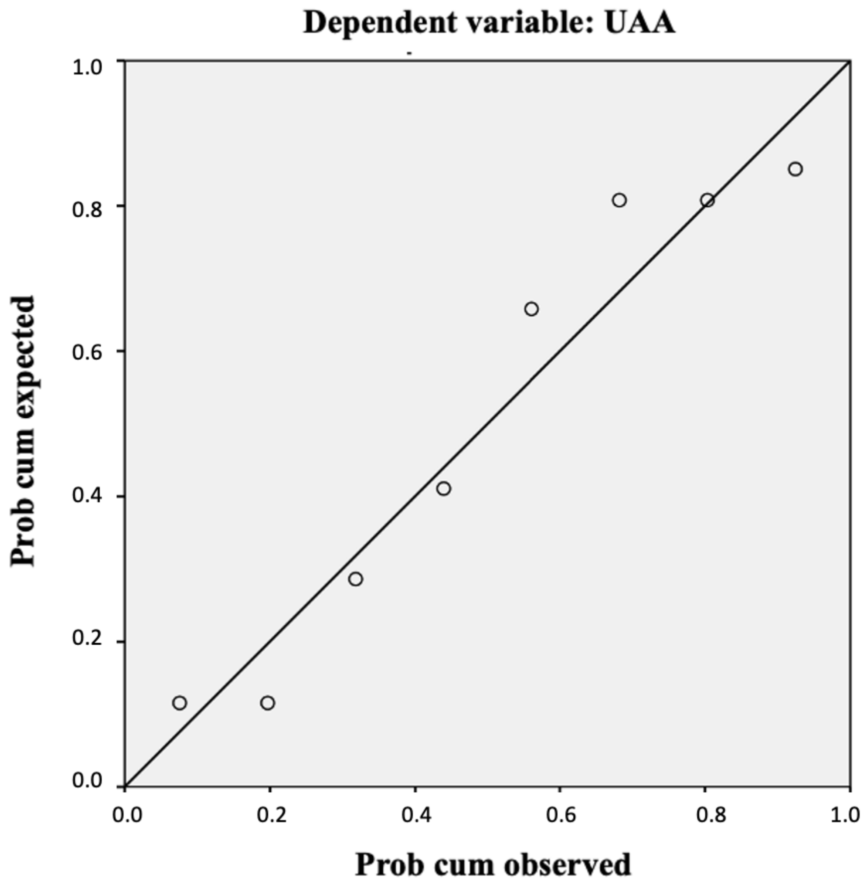

Figure 5. P-P normality graph of standardised residuals regression.

3.3. Broccoli Seed Production and Its Sustainability Levels

The experimental tests showed that the plots not treated with fertilizers (NP0) obtained a lower seed yield and germinability in both cultivars than the plots fertilized by NP1 and NP2 of the actual crop needs to be analysed. In particular the seed yield ranged from 276.3 to 1571.0 g m^{-2} in relation to the NPs and the GEs analyzed (Table 5). In particular, it was ascertained a significant interaction between the two experimental factors analysed showing for both genotypes the highest seed yield for NP2 thesis (Table 5).

Table 5. Seed yield in relation to the nutritional protocol and genotype.

	NP0	NP1	NP2	Mean
Riccio di Messina	626.9	742.8	1571.0	980.2
Sparaceddu	276.3	962.2	1087.0	775.1
Mean	451.6	852.5	1329.0	

Significance of the differences (* $p = 0.05$; *** $p = 0.001$; n.s.= no significance): Nutrition protocol (NP) ***; Genotype (GE) n.s.; Interaction NP × GE *.

In Figure 6, the seed yield shows a progressive increase from unfertilized to 50% and 100% fertilized plots, for both genotypes analysed.

The plant morpho-biometric characteristics of course supported the seed yield obtained (Tables 5 and 6). The total weight was influenced by the late transplanting date, and it varied from 30.6 to 310.7 g, respectively, for CR by N0 treatment and for SP by N2 one (Table 6). The stem diameter was significantly affected by the genotype, and it varied from 139.4 and 59.3 for SP and CR, respectively. The collar diameter was influenced significantly by both the NP, varied from 10.2 to 14.4 mm for NP0 and NP2 respectively, and genotype, varied from 7.0 to 16.6 for CR and SP, respectively. Finally, the number of the secondary branches varied significantly from 3.2 to 2.9 for SP and CP, respectively.

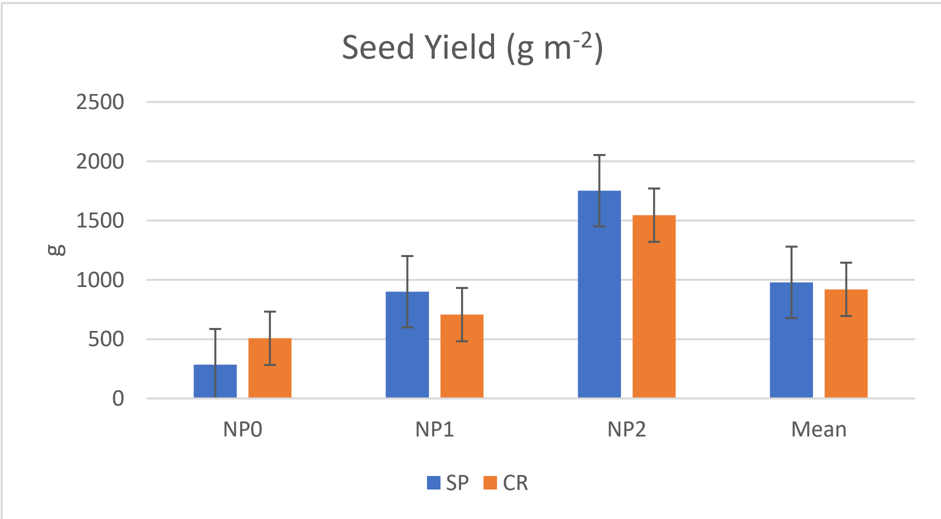

Figure 6. Seed yield per genotype and nutrition dose (the bars show the standard error).

Table 6. Main effect of genotypes (Sparaceddu SP and Cavolo Riccio di Messina CR) and nutrition protocol (NP0. NP1. NP2) on total weight, stem height, collar diameter, and branch number.

Nutrition Protocols		**NP0**			**NP1**			**NP2**		**Mean**
Characteristics	SP	CR	Mean	SP	CR	Mean	SP	CR	Mean	SP	CR
Total weight(g)	134.4	30.6	82.5	175.3	41.8	129.5	310.7	76.7	193.8	139.4	59.3
Stem height(g)	20.0	13.7	16.9	18.3	7.0	12.7	20.7	15.3	18.0	19.7	12.0
Collar diameter (mm)	14.1	6.3	10.2	15.5	6.3	10.9	20.3	8.4	14.4	16.6	7.0
Branches (n)	3.0	2.3	2.7	3.3	3.5	3.4	3.3	3.0	3.2	3.2	2.9
		Total Weight (g)			**Stem Height (g)**			**Collar Diameter (mm)**		**Branches (n)**
Nutrition protocol (NP)		***			n.s.			***		n.s.
Genotype (GE)		***			**			***		***
Interaction NP × GE		***			n.s.			n.s.		n.s.

Significance of the differences (** p = 0.01; *** p = 0.001; n.s.= no significance).

In the case of germination percentage (Figure 7), the plot fertilised with 100% showed the best results for both genotypes, however, genotype E obtained higher seed germination in IP0 than in IP1, which shows that despite the lack of fertilisation the variety analysed can produce good quality seeds. Genotype G, on the other hand, did not show any noticeable difference between unfertilised and fertilised plots, and germinability remained the same at 50% and 100% fertilisation.

In conclusion, an initial economic validation of the project idea was carried out. Even though we are aware of the need to carry out an economic validation on a larger territorial scale and on a more consistent sample of farms, Table 7 clearly shows that at the source of a 36% reduction in seed production in NP1 it is possible to achieve a cost saving of 17% compared to NP2, which can encourage entrepreneurs to adopt a more environmentally friendly attitude and in line with the EU strategy of increasing the availability of seeds suitable for organic farming.

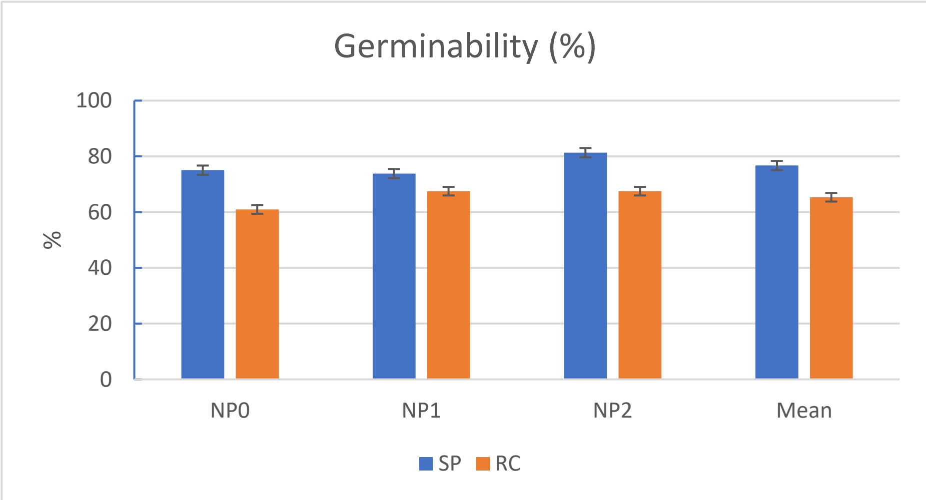

Figure 7. Germinability (%) per genotype and nutrition dose (The bars show the standard error).

Table 7. Final synthesis and validation of the experimental thesis (*).

	NP0	NP1	NP2
Materials:	2.100	3.000	3.900
—of which expenditure for fertilizers	0.0	900	1.800
Labor and services:	3.670	4.320	4.970
—of which expenditure for fertilizer administration	0.0	650	1.300
Quotas and other attributes	420	420	420
Total average cost	6.190	7.740	9.290
% variation	−33.37	−0.17	0.0
Seed yield g m^2	451.6	852.0	1.329
% variation	−66.02	−0.36	0.0
(*) Our elaboration.			

In absolute values, adopting lower levels of fertiliser use has an impact not only on material costs but also on the labour costs associated with its distribution.

4. Conclusions

The results show that agricultural production can also ensure broccoli seed of high quality through new plant nutritive tools to reduce pollutants, climate change, and negative impacts on human health. There is also a strong need to encourage growers for using new organic plant nutritive tools to achieve a result in line with the original spirit of organic farming.

So, ultimately, the research conducted confirms:

- The good combination of broccoli biodiversity and organic farming;
- The regeneration or self-reproduction of seeds by the farmer is interesting because it is stable and adequately productive.

As far as the Green Deal and the related strategies From Farm to Fork and Biodiversity 2030 are concerned, the work carried out has also confirmed that a possible increase in the area under organic management can take place starting from suitable seeds, able to produce plants with deep and branched roots that seek nourishment in the soil and, therefore, not provided in an immediate form by chemical fertilisers spread on the ground. Organic farming therefore requires the traditional broccoli landraces, i.e., linked to the characteristics of the production areas, or specifically selected for an agro-ecological practice, to be able to develop fully in fields where synthetic chemicals are not used.

It is therefore necessary to raise farmers' awareness of the implementation of more sustainable practices, specifically linked to the rational use of inputs and the use of organic fertilisers, and to demonstrate the possibility of obtaining yields that meet the farm's sustainability requirements, making the production process environmentally friendly.

The limitations of the study can be traced back to the replicability of the results to other production contexts and to other crops. Low-input production supported by the new tools for organic plant nutrition allows to reach good broccoli yield of interest for the landraces of custodian farmers, as a basis for maintaining the competitive advantage for relevant markets and stakeholders. In the future, however, multidisciplinary research must focus on the sensitivity and attitude to adopt sustainability practices and standards for the protection and conservation of biodiversity in agricultural ecosystems, as well as on the economic validation of processes and policy demands for requirements that are inconsistent with small businesses and the bureaucratic burdens that hinder their acceptance.

A further reflection should be developed beyond *B. oleracea* crops and with respect to the two sprouting broccoli typologies we are dealing with. There is a "niche" product of broccoli biodiversity, whose critical mass is extremely small, and is a product of biodiversity, still an expression of culture and territory, but with a higher consistency (at least in terms of volume of supply and of the relative market) and suitability for agroecological practices.

In the latter case, it is necessary to include all the ecological practices involved in the production of the great biodiversity expressed by the vegetable landraces and related seeds, and not just for the custodian farmers. This includes the nurseryman, who is interested in the hobby sector which represents an important commercial outlet, differentiating and supplementing its own supply to meet the demand of local vegetable amateurs and horticulturists contributing to the safeguard of traditional vegetable landraces avowing the high risk of extinction in acts. Similarly, the seed industry is paying particular attention to the traditional vegetable landraces because it is interested in their specific rusticity and adaptability in terms of soil, climate, and phytopathology, as well as their potential market interest for the organoleptic traits of the products. The use of the vegetable landraces for the constitution of commercial varieties on which to charge rights or royalties cannot be excluded.

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