



DIVERSILIENCE - Diversifying Organic Crop Production to Increase Resilience

## D 3.3 - Farmer-participatory design and assessment of warm-season legume cereal binary associations for southern Europe

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### Abstract

Cereal-legume intercropping increases biodiversity at field level, but to ensure satisfactory yield, mixtures must be optimized in terms of crop-type, species proportions and cultivation technique. Adopting a farmer-participatory approach, this research work assessed a set of promising warm-season legume-cereal mixtures for two years under organic farming in Northern Italy (cowpea and soybean-based mixtures) and in Slovenia (common bean-based mixtures) for yield ability and acceptability by farmers. In addition, promising cowpea material evaluated in Italy was tested in Slovenia for one year.

In Northern Italy, six treatments of intercropping (three legume-sorghum mixtures × two spatial arrangements) and four treatments of sole cropping were studied. The three legumes were a determinate-erect cowpea, an indeterminate-climbing cowpea, and soybean. In intercropping, sorghum and one of the legumes were sown either mixed within each row or in alternate rows. In Slovenia, binary associations of common bean with durum wheat, winter wheat and maize were studied. A first experiment addressed the effect of the interaction between two common bean varieties of indeterminate growth type and maize on grain yield, while a second experiment investigated the binary associations of two dwarf growth types of common bean varieties with winter wheat.

Intercropping cowpea and soybean with sorghum under organic farming in Northern Italy was a successful technique to sustain crop yield and at the same time increase biodiversity at the field level. Increasing sorghum sowing rate and identifying genotypes of both legumes and sorghum with a better synchrony in their phenological stages (harvest for silage and for grain) is needed to optimize the studied mixtures. Cowpea breeding for high biomass yield, less sensitivity to photoperiod and shorter reproductive period could contribute to intercropping improvement.

Associating cereals with common bean in Slovenia provided satisfactory yield for bread and winter wheat, whereas for maize the competition between the two species caused a yield reduction of maize in intercropping. Lower yield in intercropping was also measured in common bean. Further studies should address optimal variety choice and crop spatial arrangement to improve intercropping performance.

In both target regions the necessity clearly emerged of adopting techniques to reduce weed growth in the early stages of crop development to ensure the productivity of intercropping.

The evaluated cowpea germplasm in Slovenia showed high potential for organic grain production and breeding.

## Background

Improving the sustainability of cropping systems is a priority of the agriculture sector. However, maintaining land productivity with less environmental impact and at the same time promoting the provisioning of ecosystem services (including enhanced biodiversity) is challenging. In crop rotations, legume crops have key importance for organic systems due to their contribution to reducing N fertilizer through symbiotic N fixation, and to supply high-protein food or feedstuff. Intercropping is also a valuable means, together with the cultivation of variety mixtures (e.g. evolutionary population), to increase biodiversity at field level. Legume-based mixtures could also contribute to increasing resilience of cropping systems through their higher yield stability compared to monocultures. Advantages of intercropping compared to pure stands were associated with greater spatial and temporal efficiency of resource use, facilitation effects and limitation of weeds, pests and diseases (Picasso et al., 2011). However, wide adoption of multi-species mixtures is limited due to frequent yield loss compared to cultivating the most productive species in sole-crop (Li et al., 2023), possible technical challenges (crop management), and difficulties in defining optimal legume/cereal mixtures (Annicchiarico et al., 2019). Information on useful plant traits that favor the compatibility of associated species is key for successful mixtures and to guide breeding (Moore et al., 2022); however, this information is very limited for grain legumes, especially warm-season ones, while multi-species mixtures have been much less explored than binary mixtures.

#### Objectives

The aim of Task 3.3 was to co-create with farmers a set of promising warm-season legume-cereal mixtures adapted to Southern Europe environments. Phenotypic data of cowpea (*Vigna unguiculata* [L.] Walp.) and soybean (*Glycine max* [L.] Merr.) evaluated in Task 1.6, and of common bean (*Phaseolus vulgaris* L.) evaluated in Task 1.5 were used to select legume ideotypes to be used in intercropping. Identified intercropping combinations were tested for two years under organic farming in Northern Italy (cowpea and soybean-based mixtures) and in Slovenia (common bean-based mixtures). A set of promising cowpea lines evaluated in 2022 in Northern Italy were also tested for grain yield ability in Slovenia in 2024 with the aim of identify interesting genotypes for future breeding programs.

# Binary associations of sorghum with cowpea or soybean in Italy

#### Material and methods

#### Co-design of cereal-legume mixture with farmers

In winter 2022–spring 2023, a group of eight farmers were involved in the setting up of legume-cereal mixtures. Cowpea and soybean phenotypic data (Task 1.6) were discussed with farmers along with data of 11 sorghum (*Sorghum bicolor* [L.] Moench) hybrids evaluated in summer 2022 in Lodi (**Table 1**). Farmers were interested in both grain and forage (silage) production; due to uncertainty in the maturing time of cowpea (one evaluation year only), mixtures were set up with the aim of producing forage. The most important plant trait identified by farmers was plant habit, while for management they were interested in investigating different crop spatial arrangements (completely mixed stands or species sown in alternated rows). Three contrasting legume ideotypes were selected: an erect-type and a climbing-type cowpea, and a medium-tall soybean (**Table 2**). The sorghum hybrid Felsina was chosen, to match the phenological pattern of the three legumes, and because of its taller plant type.

<b>Table 1.</b> Main characteristics of the 11 tested sorghum hybrids in 2022.					
Variety	Days to	Days to	Days to	Height at	Silage yield
	flowering	doughy stage	ripening	ripening (cm)	(t DM ha⁻¹)
Arcane	42	76	97	104	18
lcebergg	43	76	97	110	21
Ggolden	44	78	97	93	20
Ruby	46	80	106	105	21
Felsina	45	80	104	143	24
lggor	44	83	101	124	21
Albita	46	83	106	137	26
PR88Y47	48	83	104	125	28
PR88Y92	51	86	106	101	17
Etrusco	49	80	104	99	19
Express	54	87	112	114	24

Plant trait	Soybean	Cowpea	Cowpea
	var. Buenos	var. IT84S-2246	var. Cp_5556
Days to flowering	30	70	68
Days to maturing	149	99	104
Habit	Erect	Erect	Climbing
Growth pattern	Semi-determinate	Determinate	Indeterminate
Plant height (cm)	94	91	81
Shoot length (cm)	94	108	215
AGB (t DM ha⁻¹)	14	9	18
Grain yield (t DM ha-1)	5	2.9	2.8
1000 seeds weight (g)	157	200	251

**Table 2.** Main characteristics of the tested legumes.

#### Field experiment

In late spring 2023, a two-year field experiment was established in Terranova dei Passerini (45°11'35"N, 9°4'47"E), under organic farming, to assess the effects of (a) legume species and plant type and (b) crop spatial arrangement on the forage yield of sorghum-legume binary associations. The experiment was designed as a randomized complete block (three replicates) and included six treatments of intercropping (three legume-sorghum mixtures × two spatial arrangements) and four treatments of sole cropping (Figure 1). In both years the following crops were cultivated: 1) sorghum, var. Felsina (Table 1); 2) a determinate-erect cowpea (EC), accession IT84S-2246; 3) an indeterminate-climbing cowpea (CC), accession Cp\_5556; 4) soybean (SOY), cultivar Buenos (Table 2). Each crop was cultivated in sole cropping (pure stand, PS) and intercropping with the two species either mixed within each row (MIX) or sown in alternate rows (ROW). In sole cropping, the sowing density was 36 seeds m<sup>-2</sup> for sorghum, 28 seeds m<sup>-2</sup> for cowpea, and 46 seeds m<sup>-</sup> <sup>2</sup> for soybean, while in intercropping the sowing density was half of that in PS for all crops. In all treatments, plots were 1.5 m wide, had six rows 0.25 m apart, and were 4.0 and 5.0 m long in 2023 and 2024, respectively. On 8 June 2023 and 15 June 2024, plots were fertilized (150 kg  $P_2O_5$  ha<sup>-1</sup>) and sown. The field was irrigated twice in both years. Treatments were harvested for forage production on 10 October 2023 and 29 October 2024.



**Figure 1.** Growth habit of the four crops (a, b), cultivated in pure stand (c), mixed rows intercropping (d), and alternate rows intercropping (e).

The dry matter (DM) aboveground biomass (AGB; t DM ha<sup>-1</sup>) was measured separately for each species and, only in 2024, for weeds. During crop growth, plant height of sorghum and legumes was measured at 48, 53, 60 and 77 days after sowing; canopy height of six plants of each species per plot was measured from the ground up to the shoot tip, or panicle tip for sorghum.

The performance of intercropping was assessed with the land equivalent ratio (LER), transgressive overyielding (TOI) and the net effect ratio (NER). The land equivalent ratio quantifies the relative land area needed in PS to provide the same yield (AGB) of 1 ha in intercropping:

 $LER = pLER_{sorghum} + pLER_{legume}$ 

pLER = AGB<sub>intercropping</sub> / AGB<sub>PS</sub>

The transgressive overyielding quantifies the relative yield obtained when 1 ha of intercropping replaces 1 ha of the most producing crop in pure stand:

TOI = AGB<sub>intercropping</sub> / max(AGB<sub>PS\_sorghum</sub>, AGB<sub>PS\_legume</sub>)

The net effect ratio quantifies the relative yield obtained in intercropping compared to the weighted mean (according to their proportion in the mixture) of the two crops:

NER = AGB<sub>intercropping</sub> / ( $P_{sorghum} \times AGB_{PS_sorghum}$ ,  $P_{legume} \times AGB_{PS_legume}$ )

Where  $P_{sorghum}$  and  $P_{legume}$  are the proportion of legume and sorghum in the mixture (both equal to 0.5 in this experiment), respectively.

Analysis of variance was used to determine the effect of experimental factors and their interactions on measured variables. Means were separated by the Bonferroni multiple comparison test.

#### **Results and discussion**

In both years emergence was not satisfactory, especially for sorghum, and forced to thin out all treatments to achieve both the correct proportion of each species in intercropping compared to pure stand and the ratio between sorghum and legumes in intercropping treatments. This resulted in lower plant density than expected in both years.

Total AGB in 2023 was 2.4–4.0 and 1.7–2.3 times higher (P<0.05) compared to 2024 in pure stand and intercropping treatments, respectively (**Table 3**). Higher rainfalls in 2024 during crop early growth (June–July) promoted weeds competition with crops and, at crop harvest, weeds accumulated a biomass of 4.7 t DM ha<sup>-1</sup> (**Table 3**). In addition, a cooler and wetter September–October period delayed crop development (and consequently harvest) in 2024. Indeed, at the 2023 harvest, cowpea and soybean were at the seed-filling stage (maturity of the two cowpea genotypes occurs scalarly, with flower, immature and mature pods at the same time), while, in 2024, few pods settled in cowpea, and soybean was at the beginning of seed-filling.

The performance of intercropping, as assessed by the LER, TOI and NER indices, depended on the year, the legume type but not on the cultivation method (mixed or alternate rows). All indices did not differ from 1.0 in 2023 (indicting no positive/negative effects of intercropping on land use and yield) while were higher than 1.0 (P<0.05) in 2024: averaged across legume types, LER was 1.72, TOI was 1.64 and NER was 1.71 (**Figure 2**).

Treatment		Crop AGB	Weeds
		t DM ha <sup>-1</sup>	
<u>Year 2023</u>			
Pure stand	Sorghum	14.8 a	n.m.ª
	Climbing cowpea	19.8 bc	n.m.
	Erect cowpea	18.5 ab	n.m.
	Soybean	19.9 bc	n.m.
Mixed rows	Sorghum + climbing cowpea	19.5 abc	n.m.
intercropping	Sorghum + erect cowpea	24.1 c	n.m.
	Sorghum + soybean	18.1 ab	n.m.
Alternate rows	Sorghum + climbing cowpea	16.0 ab	n.m.
intercropping	Sorghum + erect cowpea	19.7 bc	n.m.
	Sorghum + soybean	17.8 ab	n.m.
<u>Year 2024</u>			
Pure stand	Sorghum	5.7 abc	5.5
	CC	4.9 a	4.0
	EC	5.6 ab	4.7
	SOY	6.4 abc	4.5
Mixed rows	Sorghum + climbing cowpea	8.9 abc	4.4
intercropping	Sorghum + erect cowpea	10.5 c	4.6
	Sorghum + soybean	9.8 bc	5.6
Alternate rows	Sorghum + climbing cowpea	9.4 abc	4.7
intercropping	Sorghum + erect cowpea	9.9 bc	4.5
	Sorghum + soybean	10.1 bc	4.6
	SE	1.1	0.5
	Source	Р	Р
	Treatment	< 0.05	NS
	Year	< 0.05	-
	Treatment × year	NS	_

**Table 3.** Total crop aboveground biomass and weeds biomass measured in the pure stand and intercropping treatments in the two years.

<sup>a</sup>n.m., not measured.

The larger yield reduction in 2024 compared to 2023 in pure stand than in intercropping (**Table 3**) could explain the higher performances of intercropping in the second year. In 2023, the growing conditions for sorghum in pure stand were more favorable than in intercropping because competition with weeds was not an issue in pure stand while legumes competed well with sorghum likely because of a higher capacity to cope with a low plant density. As a result, yield reduction of sorghum in intercropping was marked (pLER = 0.28) while for legumes it was limited (pLER = 0.78). In the following year, all treatments underwent yield reduction in part due to severe competition with weeds. This affected both sorghum and legumes, and more pure stand treatments than intercropping. Therefore, the advantage of intercropping in 2024 was mostly due to a lower production

of sorghum in pure stand (pLER=0.85) rather than a lower production of legumes (pLER=0.87). Averaged across years, intercropping with the erect-type cowpea resulted in higher (P<0.05) LER (1.53), TOI (1.48) and NER (1.55) compared to those with the climbing-type cowpea and soybean (on average 1.32, 1.23 and 1.35 for LER, TOI and NER, respectively) (**Figure 2**). The erect growth habit of cowpea permitted to reach the highest biomass in both years (**Table 3**) likely due to a reduced competition with sorghum (higher pLER of the erect-type cowpea) and at the same time without causing an excessive decrease of sorghum biomass compared to other legumes (pLER of sorghum did not differ between legumes).

The vertical growth of the erect-type cowpea was not different from that of soybean, while both crops were shorter than the climbing-type cowpea, especially after it started to climb on sorghum stems ( $\approx$ 2 months after sowing) (data not shown). Therefore, the advantage of the erect-type cowpea in intercropping relied on its wider canopy compared to soybean and the climbing-type cowpea.



**Figure 2.** Performance indices of the intercropping of sorghum with a climbing-type cowpea (CC), an erect-type cowpea (EC), and soybean (SOY); means are averaged across type of intercropping (mixed and alternate rows) and year (2023 ad 2024). Different letters indicate significant differences (P<0.05) among legume type in intercropping.

# Binary associations of durum wheat, winter wheat and maize with common bean in Slovenia

At KIS, the activities for D 3.3 started in 2022 with a pilot experiment where the combination of growing durum wheat with beans (var. Topolovec) at one of the wheat growers in Krog at Murska Sobota was assessed (**Figure 4**).



Figure 4. Binary associations of durum wheat with beans (cereal-legume).

The pilot experiment showed that the assessed binary association between the two species/selected varieties (durum wheat and common bean) proved to be a successful combination on the farm. Considering the specific vegetation in the 2022 growing season (high temperatures and little precipitation), KIS still achieved an enviable yield in both groups of plants (species) in 2022.

In 2023, two intercropping experiments were carried out in Jablje (46.1453333; 14.5578667; 302 m) according to the guidelines for organic farming (**Figure 5**).



Figure 5. Photo compilation from the intercropping trials at KIS in 2023.

In the first experiment, the interaction between two common bean varieties of indeterminate growth type defined in Task 1.5 (Sortino di Trento-KIS\_5 and DG17-38-16-MVCRI\_5) and maize (an organic breeding line not yet registered as a variety) was studied.

The beans were sown at the beginning of June, when maize reached a height of about 30 cm, creating favorable conditions for the beans to use the maize as a growth support. The second experiment investigated the binary associations of two dwarf growth types of common bean varieties defined in Task 1.5 (ref: 417X316-KIS\_2 and MVCRI\_7) with winter wheat var. Patres sown in November 2022. Beans were sown between wheat rows at the end of May. Both experiments were designed as completely randomized blocks with three replications. Before sowing, representative soil samples were sent for analysis of nitrogen, phosphorus, potassium, organic matter and pH. Throughout the growing season, individual plants were monitored and evaluated according to the descriptors for each species: for beans we used the same descriptors as in Task 2.5 and for maize and wheat we used the IBPGR descriptors.

The first intercropping experiment had no effect on wheat seed mass per plot or on growth. Wheat seed mass per plot varied significantly between blocks, with the third (1.41 ± 0.20 kg) and second (1.33 ± 0.13 kg) blocks having more than double the mass of the first block (0.55 ± 0.22 kg). However, intercropping reduced the yield of the MVCRI\_7 bean. It had an average of  $1.9 \pm 1.1$  g seeds per plant in the sole crop and an average of  $0.8 \pm 0.7$ g seeds per plant in the intercrop with wheat. Higher yields were observed in KIS\_2 with an average of  $4.9 \pm 0.3$  g seeds per plant and in intercropping with wheat with an average of  $3.2 \pm 1.7$  g seeds per plant. The block had no effect on the seed yield of the bean. We also counted the number of nodules on the roots of the plants after the pods had matured. The common bean MVCRI\_7 did not produce any nodules, whereas KIS\_2 did, with an average of  $1.6 \pm 0.7$  nodules per plant in the intercrop and  $0.9 \pm 0.9$  in the monocrop. In the second intercrop trial, maize height and seed yield differed according to planting location (block). Plants in block three (195.0 ± 8.7 cm) were on average taller than plants in blocks two (183.7 ± 12.7 cm) and one (164.3 ± 9.3 cm). Accordingly, the highest average seed yield (g per plant) was obtained in block three (231.1 ± 56.3 g), followed by block one (161.9 ± 21.7 g) and block two (148.1 ± 50.3 g). Intercropping also reduced maize yields. The average yield of maize was 203.4 ± 82.0 g per plant in single-cropping, 149.0 ± 52.2 g per plant in intercropping (MVCRI\_5) and 189.1 ± 18.2 g per plant (KIS\_5). Unfavourable weather conditions (cold and rainy May) resulted in both beans and maize being sown later than would have been the case under suitable weather conditions.

Due to organic farming guidelines, no chemical weedkiller was used and a lot of weeds grew around the plants, which we removed by hand. However, by removing the weeds we exposed the beans which were then eaten by deer, especially the beans at the far end of the trial (block number three). As a result, not all the beans were able to form pods and not all the pods ripened until the first frost, so the seed yields do not reflect the actual situation. We also dug up the roots of the plants and counted the nodules, and the numbers are similar regardless of the bean variety or block. In summary, the intercropping experiments with bean and wheat showed no effect on the growth of wheat, which could be due to the shorter intercropping period. In the second experiment with maize and

beans, planting position significantly influenced maize characteristics and intercropping resulted in lower maize yields. This could be due to competition for light and space while the beans grew on top of the maize. Common bean yields were influenced by weather conditions and organic management, so in future it would be useful to limit weed growth earlier in the season.

## Evaluating cowpea for grain production under organic farming in Slovenia

Together with CREA-ZA from Italy, KIS have prepared a set of 30 cowpea genetic resources (**Table 4**) (dwarf growing habit), which were characterized and evaluated in Slovenia (and as well in Italy).

	000010000		
Name	Origin	Name	Origin
NamuesseD	Mozambik	TVu-12937	Indija
UCR_1340	/	TVu-4535	Nigerija
Chiaro di Fusignano	/	TVu-14253	Bocvana
IT83D-442	Nigerija	UCR_288	/
Pakau	Senegal	IT84S-2246	Nigerija
TVu-16278	Italija	TVu-13950	Bocvana
TVu-1609	USA	TVu-16408	Benin
Ife_Brown	/	FN-2-13-04	Mozambik
INIA-5E	Mozambik	TVu-8877	Benin
TVu-14621	Mali	Apagbaala	Gana
Bistarelli bianco	/	Mouride	Senegal
KVx_525	/	TVu-4622	Tanzanija
KVx_403-P-20-T	/	TVu-2680	Nigerija
TVu-9508	Egipt	TVu-8631	Togo
UCR_5272	/	TVu-12746	Indija

Table 4. List of cowpea genetic resources.

Five seeds of each genetic source were photographed before sowing and sowing took place at the end of May. Due to the high soil moisture, the seeds germinated poorly in the first replication and not a single seed germinated in plot 1 - IT83D-442. Despite high temperatures, the plants thrived well as the foil retained the soil moisture. There were also no pests or diseases on the plants. Most of the plants flowered in July, but some did not flower until mid-August. Despite the late flowering of some of the cowpea genetic resources, all the pods matured and the last harvesting of mature pods was carried out in the second half of September. In September and October, all the harvested material was dried, cleaned, sorted and further evaluated for post-harvest descriptors such as grain yield, 100-seed weight and seed color. Results showed that plants in the collection took

on average 8 days to emerge, 61 days to flower and 102 days to harvest (**Figure 6**). On average, 22.9 g of seeds were produced per plant, although it should be noted that the yield per plant was much higher in the case of plots where the number of plants was smaller. The weight of seeds per plot varies considerably depending on the genetic source and the number of plants thriving on it. The genetic source TVu-8877, plot number 24, had the lowest seed weight, with a total seed weight of 133.6 g from 26 plants. The genetic source TVu-16278, plot number 6, had the highest seed weight, also from 26 plants, with a total seed weight of 629.4 g from 26 plants. According to the results obtained, it can be said that cowpea has a high potential for organic production and breeding in Slovenia.



**Figure 6.** The different stages of the phenological development of cowpea plants; from emergence, growth, flowering, pod formation and harvesting of the cowpea in 2024 at KIS.

### Conclusion

Intercropping cowpea or soybean with sorghum under organic farming in Northern Italy was a successful technique to sustain crop yield and at the same time increase biodiversity at the field level. Further studies should investigate optimal sorghum sowing rates to overcome the poor emergence and crop establishment under summer conditions. A better synchronization of the phenological stages (harvest for silage and for grain) between sorghum and legumes is also a priority for mixture improvement. To this end, later sorghum genotypes should be investigated as well as earlier-maturing legumes genotypes, possibly with ad-hoc breeding programs aimed at developing high biomass yielding cowpea genotypes with less sensitivity to photoperiod and shorter reproductive period. The higher yield of the erect-type cowpea encourages a focus on non-climbing types, at least for association with conventional, grain-type sorghum (which are fairly short, <1.5 m). The type of crop arrangement did not impact the performance of intercropping; therefore, a seeder available on farm could be successfully adopted.

Associating durum wheat with common bean in Slovenia proved to be a successful combination in organic farming. Further exploration of winter wheat-common bean intercropping resulted in similar wheat yield as in pure stand, likely due to a short intercropping period; however, bean production was reduced. The depressive effect of intercropping on maize yield was noticed likely due to competition for light and space while beans grew on top of maize plants. Therefore, further studies should address optimal variety choice and crop spatial arrangement to improve intercropping performance.

For all cereal-legume intercropping tested in Northern Italy and Slovenia, adopting techniques to reduce weeds growth in the early stages of crop development emerged as a priority.

Cowpea showed high potential for organic grain production and breeding in Slovenia.

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