

EU CAP Network Focus Group 'Crop associations including Milpa and protein crops'

Mini Paper 1

Cultivar testing is key to boost uptake of crop associations in breeding and farming

Pierre Hohmann (Coord.), Sebastian Kussmann, Martin Bourke, Essaid Ait Barka, Christian Schöb, Alain Baranger, Diego Rubiales, Paolo Annicchiarico

September 2024







Disclaimer

This Mini Paper has been developed within the frame of the EU CAP Network Focus Group 'Crop associations including milpa and protein crops' with the purpose of providing input to the Focus Group discussions and final report.

The information and views set out in this Mini Paper are those of the author(s) and do not necessarily reflect the official opinion of the Commission. The Commission does not guarantee the accuracy of the data included in this Mini Paper. Neither the Commission nor any person acting on the Commission's behalf may be held responsible for the use which may be made of the information contained therein.

If you wish to cite this Mini Paper, please refer to it as 'Annex to the <u>final report of the EU CAP</u> <u>Network Focus Group 'Crop associations including milpa and protein crops'</u>, 2024'.



Abstract

Breeding, cultivar testing and cultivar information systems are key factors for the successful implementation of crop associations (CA) in farming systems. However, many breeders are still hesitant to incorporate cultivar selection for mixing ability in their breeding schemes because of its additional cost/efforts and the small market size of CA. The inclusion of mixture performance in existing and new cultivar testing schemes may leverage crop associations in breeding and farming. Breeders will have a first recognition of their efforts to select for mixing ability, and seed marketing and farmers will get access to necessary information for the selection of cultivars for CA. Here, challenges, opportunities and solutions for CA-inclusive cultivar testing are assessed (see highlights in **Box 1**). Cultivar testing and information systems for CA can be an important leverage point to boost CA in breeding, farming, and along the agricultural value chain. Challenges however remain and some dedicated efforts and collaborations between farmers, breeders, variety testing and seed registration bodies, scientists and public administrations are required to implement the proposed changes.

Box 1 Key message on using cultivar testing to boost CA. Source : European Commission

Key problems

Farmers:

- Lack of access and knowledge of suitable varieties for CA
- Need of guidelines how to choose cultivars for CA

Breeders:

- Difficulties to register cultivars specifically suitable for CA
- Lack of incentives to make additional efforts for narrow markets

Registration bodies and extension services:

- Lack of knowledge and additional resources needed to test for CA
- Current regulatory frameworks are not aligned with CA-relevant specifications

Key opportunities

- Include CA performance in official variety registration trials:

 Use pure stand key traits (e.g. related to competitive
 - ability) as proxies for CA performance b. Include CA in variety trials by choosing a suitable,
 - competitive tester and possibly pre-select varieties based on trait assessments (see 1a)
- 2. Use cultivar information from pure stand testing and guidelines to choose cultivars for CA:
 - a. Keep data resources alive and up-to-date
 - b. Connect different data resources
 - c. Develop digital tools for user-friendly access and decision-making
- 3. Ensure seed availability and local communication of CAsuitable varieties:
 - a. Databases like organicXseeds serve as example
- 4. Include CA in private variety trials (such as living labs and farm networks) as vessel for participatory cultivar testing:
 - a. Successful examples of LL and participatory apps like SeedLinked exist

Introduction

A shift in crop production is necessary to secure crop and food production through diversification at all levels, including crop cultivars with traits for diverse farming systems such as cultivation in crop associations, i.e. any form of bi- or multi-species mixtures in co- (withand between-row), relay-, strip-intercropping or agroforestry (agrosilvicultural, silvopastoral, agrosilvopastoral) including Milpa and protein crops as cash, cover, nurse and companion crops. In the EU, policies towards making agriculture more sustainable through the provision of ecosystem services (management of nutrients and resources, weed, pest and pathogen control etc.) take precedence over the predominant focus on increased yields (Stomph et al. 2020). Breeding has been recognised as a crucial element to optimise services and yields for CA (Annicchiarico et al. 2019). However, more holistic plant characterisation like testing for suitability for cultivation in CA are not yet considered in cultivar registration and testing schemes. Thus, (i) growers have limited access to information about cultivars suitable for



cultivation in CA and (ii) breeders face difficulties to register varieties specifically developed for CA (Rubiales et al. 2023). Research in the field of CA led to substantial progress on why and how to breed for mixture performance (Annicchiarico and Proietti 2010; Haug et al. 2023; Litrico and Violle 2015). The uptake of such knowledge in breeding programmes is nevertheless hampered by the currently low demand by farmers and complexity of crop association breeding. Here, we describe what we perceive as the most important challenges and opportunities, and propose solutions to integrate mixture performance in cultivar testing and registration both seen as a key element to increase awareness and help farmers to choose suitable varieties as well as providing incentives for breeders to integrate mixing ability in their breeding programmes.

Challenges and opportunities

This section deals with methods to characterise species and varieties for their suitability for cultivation in CA. Two approaches are considered: (i) the combination of cropping partners (two or more species) based on existing knowledge on mixing ability and competition dynamics and (ii) the incorporation of mixing ability in official variety testing.

1. Testing and characterisation of cultivars for crop associations

The number of possible combinations of species and varieties in CA is almost endless. Therefore, specific tests must be performed to characterise cultivars for their suitability in the mixtures. There is a potentially huge number of possible companion varieties for a focus variety evaluated for suitability for CA, in bispecific as well as multispecies mixtures. This situation is further complicated by the possible impact on competition dynamics of different crop managements with respect to inclusion in the crop rotation, nitrogen fertilisation levels, sowing rates of each component, sowing patterns and end uses.

General key questions for cultivar testing are, in this context, the following ones:

- A. Is the performance of a given cultivar consistent enough between pure and mixed stands (implying modest effort on variety evaluation for CA)?
- B. Are there key varietal traits to optimise mixing ability that deserve to be assessed in mixture and/or pure stand cropping conditions?
- C. Is the mixing ability of a cultivar a fairly general characteristic or does it largely depend on the companion species and genotype (implying, in the latter case, substantial effort on variety evaluations for each crop species/genotype)?
- D. What are the effects of crop management and/or pedo-climatic environments on the mixing ability of a cultivar (possible implications on testing environment/management practice)?

Information generated by decades of earlier research (with an historical emphasis on legumegrass perennial forages) can be crucial, to identify general or likely response patterns of crop genotypes that can help design cost-efficient variety evaluation strategies (Annicchiarico et al. 2019). It is for forages where we identified a successful example of an official variety testing scheme that considered mixture performance (see summary of an interview with Dr. Daniel Suter, Head of Forage Crop Variety Testing in Switzerland, **Box 2**). This example provides valuable insights that can help (i) to implement mixture testing in other European countries and (ii) to extrapolate know-how to other cultivation systems such as arable farming.

Box 2 Summary of an interview with Dr. Daniel Suter, Head of Forage Crop Variety Testing in Switzerland. *Source : European Commission*

For over fifty years, Switzerland has tested forage crop varieties not only in monoculture but also in mixed stands, primarily to understand the competitive strength of these varieties. This is particularly important for formulating



clover-grass mixtures, which are used in over 90% of Swiss meadows and pastures. An interview with Dr. Daniel Suter, Head of Variety Testing for Forage Crops in Switzerland, explored the intricacies of this testing process and its broader implications. The full interview is published in xxx. Key points from the interview are outlined as follows:

Selection of mixture partners

- Any recommended variety with a minimum level of competitive ability can be a mixture partner (tester)
- Trials focus on simple mixtures (binary to ternary) to gauge the competitive strength of tested varieties

Plant traits

- The primary trait assessed in mixtures is the yield proportion of the tested variety
- Information on early maturity is also considered for mixture suitability.

Participation of stakeholders

- Forage variety trials in Switzerland are primarily conducted by a public institute (Agroscope)
- Mixture testing influences breeding goals
- The list of recommended varieties aids seed merchants and developers in creating optimal mixtures (as opposed to farmers directly as would be the case for e.g. arable farming)
- The Swiss standard mixture system mandates the use of recommended varieties for quality labeling

Other success factors

- Effective agronomic reasoning is crucial for establishing regulatory frameworks
- The system should be self-sustaining, without relying on subsidies
- Communication tools and efficient dissemination of results are important, but the experience is limited to forage crops
- There might be a lack of knowledge or resources for mixture testing in other crops, but taking initial steps is essential.

Conclusion

Dr. Suter emphasizes the importance of understanding competitive strength in forage crop varieties and the impact of this knowledge on breeding and agricultural practices. The Swiss approach to variety testing in mixed stands offers valuable insights that could be adapted by other countries and applied to different cultivation systems.

Unfortunately, experiments that compared the performance in pure stand and in binary mixture of relatively large arrays of genotypes/cultivars belonging to different crop species or types have been quite infrequent. Various experiments by Annicchiarico et al. (1994, 2021) on white clover/forage grass and pea/cereal mixtures indicated a pattern of competition dynamics where the genotype performance depended on the level of competitive ability under severe competition. Already Harper (1977) emphasised the importance of improving the weaker partner of an association to increase the yield efficiency of the mixture. Further, total CA can be optimised by nil or very reduced N fertilisation or increased legume sowing rate for annual (e.g., Hauggaard-Nielsen and Jensen, 2001; Kiwia et al., 2019) and perennial crops (Zannone et al., 1986).

Importantly, the above results indicate that variety evaluation for CA is all the more important when the focal species tends to be outcompeted because of its intrinsically low competitive ability or of the adopted crop management. Studies reviewed by Annicchiarico et al. (2019) and recent findings for pea/barley (Haug et al., 2023) and grain legume/wheat (Moutier et al., 2022) indicated the much larger general compared to specific mixing ability effects, confirming that suitability of a variety for CA follows some general features. These findings facilitate mixture cultivar testing because they suggest that (i) not all possible (specific) combinations have to be tested and (ii) only a few varietal testers are needed to determine the mixing ability of a given cultivar. The importance of the competitive ability of a given companion was also highlighted for the Swiss forage variety testing example (see interview summary in **Box 2**). An alternative approach (for when there is more than one tester) employs incomplete factorial designs (as shown by Haug et al. 2021) in an attempt to test a reduced number of combinations of various cultivars on both sides, and to infer from statistical models the assessment of non-studied combinations. Whenever specific mixing ability is low (as shown for pea/barley



mixtures), incomplete factorials can drastically improve genetic gain by testing an increased number of genotypes using the same amount of resources.

Lastly, CA performance and services highly depend on environments. A trial network of 8 wheat varieties combined with 5 grain legume (pea and faba bean) tester varieties across 9 environments (3 sites x 3 years) showed that both the global yield and species respective mixing abilities were highly dependent upon environments (Moutier et al., 2022). This leads to the consideration (i) that some varietal combinations may be more adapted in specific pedoclimatic and management conditions, and (ii) that breeding may have to target specific management/environmental conditions to be successful.

2. Variety registration - Current developments and open questions

Concerns about seed regulations and resulting variety registration and testing regimes question the relevance of the conventional variety trials for diverse farming systems in Europe (Chable et al. 2011). This is particularly relevant for cultivars specifically developed for CA as their performance differs between cultivation in mixed and pure stand. Their yield potential for CA cannot be concluded from testing in pure stand - well performing cultivars in CA are not necessarily performing well in pure stand and vice versa (Haug et al. 2023). The subsequent rejection of cultivars developed for CA in the official variety registration tests potentially has negative consequences for the sustainability and productivity of agriculture in the EU, as cultivars developed for CA cannot enter the market. In the current proposal for a new EU Seed law, sustainability traits are integrated in the testing. It is however not yet defined what sustainability traits are, how they may be prioritised and taken into account in the registration process together with yield performance data. Testing the CA suitability of cultivars would be a relevant sustainability trait.

Each cultivation system (e.g. conventional and organic farming) has its own priorities concerning traits and therefore variety development and testing need to be target system-specific (Lammerts Van Bueren et al. 2002, Lammerts Van Bueren et al. 2011). Similarly, for CA, cultivar development requires a systems approach, from the decision to breeding for CA to the final stages of variety testing and release (Moore et al. 2022). It deals with (i) defining relevant traits for diversification of agriculture, (ii) the need to consider the end use in the evaluation of the benefits, and (iii) identifying the traits that might be desirable for different actors along the value chain. Moore et al. (2022) propose a model of how to organise breeding and variety testing for CA and how mixing ability can be taken into account in the breeding process (**Figure 2**).



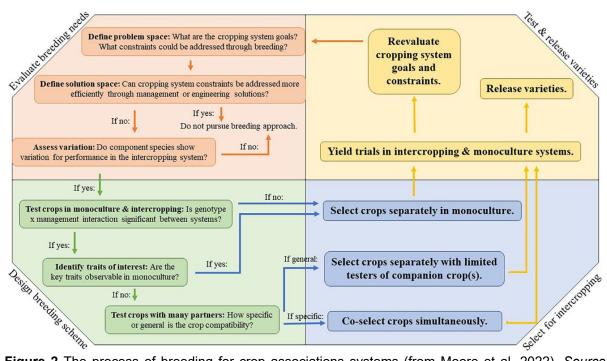


Figure 2 The process of breeding for crop associations systems (from Moore et al. 2022). Source: European Commission

If key traits (and adequate levels) determining the suitability for CA are known, selection in the breeding process can be based on these traits in monoculture. If key traits are unknown, selection of the target crop in CA is necessary (see decision paths in the green and blue sections of Figure 2). In all cases, testing for mixing ability is required during final cultivar testing and registration (see yellow section of Figure 2).

Private and public variety development and public variety testing and registration are complex and cost-intensive. Processes including specific tests for sustainability traits such as mixing ability require specific trials to characterise varieties for relevant traits in target environments to permit their marketing. These can be performed by (i) integrating specific variety trials into the existing public testing schemes (official public variety testing) or (ii) establishing new testing schemes or iii) officially recognising results in official testing schemes from non-recognised agronomic variety trials (universities, agricultural research institutions, participatory on-farm trials etc., see also subchapter 'Digital Solutions'). In the context of the currently small proportion of CA practiced by farmers, the question is how "demand" can be created and which actors create incentives: Plant breeders and cultivar holders ask for registration and official variety testing for CA and/or public testing agencies perform CA trials to provide information about variety CA performance thus stimulating breeders to breed for CA.

Another issue is related to seed availability and variety information. Farmers are not always always aware of unofficial variety trials in their or neighbouring region. And even if they were able to identify a suitable variety, information of available seed can be difficult to obtain, especially from neighbouring countries. The database <u>organicXseeds</u> serves as an example of how to improve information on the availability of seed beyond borders. The database allows seed suppliers to register and put their offers and seed availabilities on the web portal, and farmers can then verify the seed availability, directly contact suppliers, and apply for individual licences. While organicXseed focuses on organic seeds and aims to alleviate the seed derogation issue, such a database could be adapted to serve farmers (also conventional ones) for other purposes such as finding seed of CA-suitable varieties for their region.



Proposed solutions

1. <u>The Living Field Lab approach</u>

Multi-actor collaboration and multidisciplinary research play a pivotal role for the implementation of CA-based farming practices (Hauggaard-Nielsen et al. 2021). The collaborative efforts of researchers showcase the benefits of bringing together expertise from various fields, such as agronomy, ecology, and genetics (Brooker et al. 2015; Wolfe et al. 2021). This interdisciplinary approach allows for a more holistic understanding of crop associations and enables researchers and other stakeholders to address challenges and opportunities from different perspectives.

The Living Field Lab Approach provides such a multi-actor, multi-methodology solution bringing together relevant actors of the agricultural value chain and exploring on-farm potential suitability of cultivars for various crop associations (see examples in **Box 3**). Living labs are collaborative environments where real-world conditions are used to test and develop new technologies, products, or solutions. In the context of agriculture, on-farm research trials within living labs involve conducting experiments or tests directly in a farm setting, often in collaboration with farmers, researchers, and technology developers. Living labs provide a valuable bridge between theoretical research and practical application by directly involving end-users (farmers) in the research and development process. They foster innovation, address real-world challenges, and facilitate the adoption of sustainable and efficient agricultural practices.

Collaboration between field living labs and official variety trialling institutes could allow for the identification and further examination of promising cultivars for CA. This collaboration would ensure that the cultivars tested in field living labs underwent rigorous evaluation and validation, leading to their potential adoption by farmers.

Box 3 Examples on the use of living labs to progress cultivar testing for crop associations. Source : European Commission

Example one

One successful example of a living lab conducting crop variety trials is the Living Field Lab in the UK. This initiative, led by the Innovative farmers network and the Organic Research Centre, engages farmers, researchers, and other stakeholders to experiment with and test different crop varieties in real farming conditions. They conduct trials on various crops, such as wheat, barley, oats, and legumes, testing different varieties for traits like yield, resilience to diseases, adaptability to changing climate conditions, and nutritional value. The group has been involved in various CA trials aimed at exploring the benefits of planting different crops together. The trials take place on participating farms across different regions, allowing for a broader understanding of how these varieties perform in various environments.

The Living Field Lab encourages collaboration and knowledge-sharing among farmers, researchers, and experts, aiming to improve agricultural practices, sustainability, and crop resilience while considering farmers' needs and experiences. It's a great example of a living lab that facilitates practical experimentation and innovation in agriculture. The group emphasises open sharing of findings, successes, and failures. Farmers share their experiences, data, and insights gained from the Field Labs with each other, researchers, and the wider community. This knowledge exchange helps refine practices and build a robust knowledge base.

Example two

The field living lab approach was also adopted in the DiverIMPACTS project. DiverIMPACTS was a large-scale European research project focused on promoting crop diversification strategies, including CA, to enhance the sustainability and resilience of agricultural systems. The project involved collaboration between researchers, farmers, agricultural advisors, industry stakeholders, and policymakers across multiple countries in Europe. The field living lab approach within the DiverIMPACTS project involved establishing experimental plots on participating farms, where various cultivar combinations were tested under on-farm conditions. These living labs served as platforms for participatory research, where farmers and researchers worked together to design, implement, and monitor CA cultivar trials. This approach allowed for the integration of scientific knowledge with practical on-farm experience and facilitated the co-creation of knowledge between researchers and farmers. Through the field living lab approach in the DiverIMPACTS project, a wide range of CA systems were evaluated, including different combinations of main crops and companion crops, as well as diverse management practices. Data on crop



performance, yield, weed suppression, pest control, soil health, and economic viability were collected from these trials, providing valuable insights into the potential benefits and challenges of CA.

2. Digital solutions

Creating digital solutions for assessing the suitability of cultivars for CA and assembling information from crop association trials offer several benefits. Digital solutions streamline the process of collecting, organising, and analysing data.

By assembling information from various trials, digital solutions can provide a comprehensive overview of how different cultivars perform in diverse conditions. This helps in understanding the adaptability and performance of varieties in various CA scenarios.

With the aid of analytics and data visualisation tools, these solutions can offer insights and recommendations to farmers or researchers, aiding in selecting the most suitable varieties for CA based on specific criteria, such as yield, compatibility, or resilience to environmental factors.

Digital platforms can facilitate easy access to trial data and information, promoting collaboration among researchers, agronomists, and farmers. This accessibility can lead to a broader understanding of cultivar performance in different crop associations.

As more data becomes available, digital systems can adapt and refine their recommendations, ensuring that information remains relevant and up-to-date.

Creating a digital database for crop associations would involve steps such as:

- <u>Research and data collection</u>: Gather information on various cereal and legume varieties suitable for CA. This involves collecting data on their characteristics, growth habits, compatibility, yield potential, disease resistance, and nutritional value.
- <u>Database structure</u>: The database structure could be organised by crop types, varieties, characteristics (such as growth patterns, maturity dates, cultivar height, nutrient requirements, etc.), and compatibility with other crops.
- <u>Digital platform development</u>: Create a user-friendly digital platform or interface to access and search the database. This could be a website, an app, or software specifically designed for farmers, advisers and researchers. Implement filters and search options within the database. This allows users to easily find specific varieties based on criteria like CA combinations, region, climate, soil type, or desired characteristics (see examples in **Box 4**).

Box 4 Examples of existing digital solutions that can be directly used or adapted to progress cultivar testing for crop associations. *Source : European Commission*

Example one

One example of an app for conducting participatory on-farm cultivar trials is <u>SeedLinked</u>. The app offers userfriendly interfaces for setting up, designing, managing, evaluating and disseminating trial results from decentralised field trials. SeedLinked could be used for on-farm variety trials to test varieties for their performance in CA.

Example two

In order to ensure harmonisation and effective management of data, a data template such as that developed under the <u>DIVERSify</u> project could be used. In the DIVERSify project, a decision support tool was developed to examine mixing ability of different crop species combinations, management options and climatic regions. DIVERSify also designed a standardised Microsoft Excel file to facilitate data collections of CA trials.

Example three

For cover crops and living mulches, an interactive web tool was developed that combines a comprehensive Wiki on all aspects of diversification in agriculture, a Decision Support Tool, and a Species Database: <u>AgroDiversity</u> <u>Toolbox</u>.

Any database or digital tool needs to stay updated with new cultivars, research findings, and user feedback. Continuous improvement is vital to maintaining its usefulness. Collaboration



between agricultural experts, farmers, and researchers is crucial for the success of such a database. It should address the practical needs and challenges faced by those involved in CA practices. Overall, digital solutions for assessing variety suitability for CA and collating trial information offer a transformative way to leverage data and technology for more efficient, informed, and sustainable agricultural practices.

3. <u>Cultivating diversity: incentives and support for sustainable crop</u> practices

Promoting crop associations (CA) among farmers and breeders requires a multifaceted approach with incentives and support mechanisms. Financial incentives, like subsidies and tax benefits for sustainable practices, make diverse cropping systems economically viable. Breeders benefit from research grants and collaborative incentives, fostering innovation and knowledge exchange. Recognition and certification programs add credibility, giving market advantages to those adhering to sustainable practices. Awards celebrate innovation, inspiring others. Market access is crucial, offering premium prices for diversified crops and ensuring stable markets through supply chain collaboration. Specialised training and extension services empower farmers with knowledge and support. Demonstration farms and online platforms showcase successful practices and facilitate peer-to-peer learning.

Evaluating the long-term impacts of crop associations involves assessing ecological effects (soil health, biodiversity, pest management, water-use efficiency) and economic impacts (yields, costs, market stability, labour). Social impacts include farmer perspectives, job creation, food security, and community cohesion. Knowledge transfer initiatives must be assessed for their impact on agricultural sustainability. Long-term monitoring programs and modelling tools are essential to track and project sustainability indicators in crop association systems.

4. The use of key traits in cultivar testing for crop associations

Choosing species to assemble mixtures is usually based on their potential ability (i) to show some complementarity and reduced competition in the use of resources (e.g. water, light, nutrients) in space and/or time, (ii) to compensate in case of damages to one of the component species, and (iii) to cooperate to generate favourable cropping conditions (Justes et al, 2021). The choice also considers feasibility and ease in the cropping process (sowing, management and harvesting procedures) in order to finally optimise performance per unit of land area and provision of ecosystem services. Plant traits allowing this optimisation (such as canopy height, growth dynamics, phenology, root architecture as components of competitive ability against weeds or companion crops, efficient resource utilisation, reduced susceptibility to allelopathy, and ability to control biotic and abiotic stresses) often show a high within-species variation, both at the plant and at the canopy scales. The choice of adequate varietal traits is therefore crucial to this optimisation both in production and breeding (Demie et al, 2022), as well as the knowledge of the plasticity of these traits across scales (isolated plant, intraspecific in sole crops or interspecific population in mixture scales). Predictability of traits between scales highly depends upon complexity of their genetic control, heritability and interactions with both management procedures and local pedoclimatic conditions (Kammoun et al, 2021). Identifying traits and trait combinations, and producing recommendations on experimental designs and statistical procedures, are still fields that need exploration, both for breeding and variety certification. This may highly depend upon the objective: with the purpose of breeding or producing a focus variety/species as an ideotype, one may consider the companion variety as part of the global environment, whereas with the objective to produce an optimised performance of the variety combination as an ideomix, one will consider variety couples interacting with environments.

Trait expressions of crops differ in mixtures vs monocultures, demonstrating that currently available commercial varieties bred for monoculture cropping are only suboptimal to exploit

the full benefits of CA (Stefan et al. 2022). Specifically, research has shown that plants in mixtures grow taller, have lower leaf dry matter content and higher specific leaf area, tend to spatially differentiate their root distribution and therefore belowground water uptake (Schmutz & Schöb 2023). This leads to improved niche differentiation and consequently to more yield (Engbersen et al. 2022). Trait complementarity, including facilitation traits (Schöb et al. 2018) is selected for in mixtures, providing opportunities to improve existing breeding material for high mixture performance through evolutionary plant breeding (Stefan et al. 2022; López-Angulo et al. 2023). Cultivars with traits reflecting more exploitative growth strategies might show better general mixing ability and therefore be better suited for use in CA. As a consequence, cultivar testing for mixing ability could be facilitated by assessing identified key traits expressed in mixture.

Nevertheless, morphological and agronomic traits observed in pure stand that are associated with greater competitive ability or compatibility with associated species could also be used as a preliminary indication of suitability for CA in cultivar testing and, possibly, for the definition of a performance index aimed to predict the performance for CA of a focal variety grown in pure stand. Taller plant stature (which is associated with higher relative growth rate) is reportedly a key trait for competitive ability of plants in general (Wisheu & Keddy, 1992) and crop plants in particular (Annicchiarico et al., 2019). For pea-barley mixtures, stipule length, onset of flowering and early vigour were identified as suitable pure stand key traits of pea that predict mixing ability (Haug et al. 2023). There might even be possibilities to use genetic markers of the plants and their plant-associated microbiome as a useful mixing ability trait, e.g. as recently shown by Cadot et al. (2023) and Wuest et al. (2023). Other intrinsic characteristics (as displayed in pure stand) have been envisaged (Litrico and Violle, 2015), but their impact on the suitability for CA has been less consistent and more elusive (Annicchiarico et al., 2019). Prediction indices for mixing ability e.g. based on the combination of plant height and pure stand performance proved valuable for white clover/forage grass (Annicchiarico, 2003) and pea/cereal mixtures (Annicchiarico et al., 2021).

In conclusion, through deliberate variety testing and breeding strategies targeting these traits, crop cultivars can be identified or developed to thrive in diverse cropping systems, contributing to improved productivity, profitability, and sustainability in agriculture. The integration of mixing ability in cultivar testing can be facilitated either by assessing key traits in pure stand as an easy-to-implement proxy of mixture performance or by assessing key traits directly in mixture as a more reliable indicator. In either case, it is vital to first identify and then make available data of such key traits.



References

- Annicchiarico, P. (2003). Breeding white clover for increased ability to compete with associated grasses. *The Journal of Agricultural Science*, *140*(3), 255–266. https://doi.org/10.1017/S0021859603003198
- Annicchiarico, P., Collins, R. P., De Ron, A. M., Firmat, C., Litrico, I., & Hauggaard-Nielsen, H. (2019). Do we need specific breeding for legume-based mixtures? *Advances in Agronomy*, *157*, 141–215. https://doi.org/10.1016/BS.AGRON.2019.04.001
- Annicchiarico, P., Nazzicari, N., Notario, T., Monterrubio Martin, C., Romani, M., Ferrari, B., & Pecetti, L. (2021). Pea Breeding for Intercropping With Cereals: Variation for Competitive Ability and Associated Traits, and Assessment of Phenotypic and Genomic Selection Strategies. *Frontiers in Plant Science*, *12*, 731949. https://doi.org/10.3389/FPLS.2021.731949/BIBTEX
- Annicchiarico, P., & Piano, E. (1994). Interference effects in white clover genotypes grown as pure stands and binary mixtures with different grass species and varieties. *Theoretical and Applied Genetics*, *88*(2), 153–158. https://doi.org/10.1007/BF00225891/METRICS
- Annicchiarico, P., & Proietti, S. (2010). White clover selected for enhanced competitive ability widens the compatibility with grasses and favours the optimization of legume content and forage yield in mown clover-grass mixtures. *Grass and Forage Science*, 65(3), 318–324.
- Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., Hawes, C., Iannetta, P. P. M., Jones, H. G., Karley, A. J., Li, L., Mckenzie, B. M., Pakeman, R. J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C. A., Zhang, C., ... White, P. J. (2015). Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, *206*(1), 107–117. https://doi.org/10.1111/NPH.13132
- Cadot, S., Hohmann, P., Hsung, M.-H., Hartmann, M., Haug, B., Wille, Dr. L., Messmer, M., & Bodenhausen, N. (2023). Fungal microbiome indicators are associated with genotypic variation in pea root rot susceptibility when intercropped with barley. *Phytobiomes Journal*. https://doi.org/10.1094/PBIOMES-07-23-0066-MF
- Chable, V., Louwaars, N., Hubbard, K., Baker, B., & Bocci, R. (2011). Plant Breeding, Variety Release, and Seed Commercialization: Laws and Policies Applied to the Organic Sector. *Organic Crop Breeding*, 139–159. https://doi.org/10.1002/9781119945932.CH8
- Demie, D. T., Döring, T. F., Finckh, M. R., van der Werf, W., Enjalbert, J., & Seidel, S. J. (2022). Mixture × Genotype Effects in Cereal/Legume Intercropping. *Frontiers in Plant Science*, *13*, 846720. https://doi.org/10.3389/FPLS.2022.846720/BIBTEX
- Engbersen, N., Stefan, L., Brooker, R. W., & Schöb, C. (2022). Using plant traits to understand the contribution of biodiversity effects to annual crop community productivity. *Ecological Applications*, *32*(1), e02479. https://doi.org/10.1002/EAP.2479
- Harper,J.L.(1977).Populationbiologyofplants.892.https://books.google.com/books/about/Population_Biology_of_Plants.html?id=q1EVAQAAIAAJ
- Haug, B., Messmer, M. M., Enjalbert, J., Goldringer, I., Flutre, T., Mary-Huard, T., & Hohmann, P. (2023). New insights towards breeding for mixed cropping of spring pea and barley to increase yield and yield stability. *Field Crops Research*, 297. https://doi.org/10.1016/j.fcr.2023.108923
- Haug, B., Messmer, M. M., Enjalbert, J., Goldringer, I., Forst, E., Flutre, T., Mary-Huard, T., & Hohmann, P. (2021). Advances in Breeding for Mixed Cropping – Incomplete Factorials and the Producer/Associate Concept. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.620400
- Hauggaard-Nielsen, H., & Jensen, E. S. (2001). Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N availability. *Field Crops Research*, 72(3), 185–196. https://doi.org/10.1016/S0378-4290(01)00176-9
- Hauggaard-Nielsen, H., Lund, S., Aare, A. K., Watson, C. A., Bedoussac, L., Aubertot, J.-N., Chongtham, I. R., Bellostas, N., Topp, C. F. E., Hohmann, P., Jensen, E. S., Stadel, M., Pinel, B., & Justes, E. (2021). Translating the multi-actor approach to research into practice using a workshop approach focusing on species mixtures. *Frontiers of Agricultural Science and Engineering*, 8(3). https://doi.org/10.15302/J-FASE-2021416
- Justes, E., Bedoussac, L., Dordas, C., Frak, E., Louarn, G., Boudsocq, S., Journet, E. P., Lithourgidis, A., Pankou, C., Zhang, C., Carlsson, G., Jensen, E. S., Watson, C., & Li, L. (2021). THE 4C APPROACH AS A WAY TO UNDERSTAND SPECIES INTERACTIONS DETERMINING INTERCROPPING PRODUCTIVITY. *Frontiers of Agricultural Science and Engineering*, *8*(3), 387–399. https://doi.org/10.15302/J-FASE-2021414
- Kammoun, B., Journet, E. P., Justes, E., & Bedoussac, L. (2021). Cultivar Grain Yield in Durum Wheat-Grain Legume Intercrops Could Be Estimated From Sole Crop Yields and Interspecific Interaction Index. *Frontiers in Plant Science*, *12*, 733705. https://doi.org/10.3389/FPLS.2021.733705/BIBTEX
- Kiwia, A., Kimani, D., Harawa, R., Jama, B., Sileshi, G. W., Khalid, K., Al, S., Al, N., & Dist, Y. (2019). Sustainable Intensification with Cereal-Legume Intercropping in Eastern and Southern Africa. *Sustainability 2019, Vol. 11, Page 2891, 11*(10), 2891. https://doi.org/10.3390/SU11102891
- Lammerts Van Bueren, E. T., Jones, S. S., Tamm, L., Murphy, K. M., Myers, J. R., Leifert, C., & Messmer, M. M. (2011). The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. *NJAS Wageningen Journal of Life Sciences*, *58*(3–4), 193–205. https://doi.org/10.1016/J.NJAS.2010.04.001

Lammerts van Bueren, E. T., Struik, P. C., & Jacobsen, E. (2002). Ecological concepts in organic farming and their consequences for an organic crop ideotype. *NJAS - Wageningen Journal of Life Sciences*, *50*(1), 1–26. https://doi.org/10.1016/S1573-5214(02)80001-X

Litrico, I., & Violle, C. (2015). Diversity in Plant Breeding: A New Conceptual Framework. *Trends in Plant Science*, 20(10), 604–613. https://doi.org/10.1016/J.TPLANTS.2015.07.007

- López-Angulo, J., Stefan, L., Engbersen, N., & Schöb, C. (2023). Ecological and evolutionary effects of crop diversity decrease yield variability. *Journal of Ecology*, 111(6), 1242–1253. https://doi.org/10.1111/1365-2745.14092
- Moore, V. M., Schlautman, B., Fei, S. Z., Roberts, L. M., Wolfe, M., Ryan, M. R., Wells, S., & Lorenz, A. J. (2022). Plant Breeding for Intercropping in Temperate Field Crop Systems: A Review. *Frontiers in Plant Science*, *13*, 843065. https://doi.org/10.3389/FPLS.2022.843065/BIBTEX
- Moutier, N., Baranger, A., Fall, S., Hanocq, E., Marget, P., Floriot, M., & Gauffreteau, A. (2022). Mixing Ability of Intercropped Wheat Varieties: Stability Across Environments and Tester Legume Species. *Frontiers in Plant Science*, *13*, 877791. https://doi.org/10.3389/FPLS.2022.877791/BIBTEX
- Rubiales, D., Enjalbert, J., Hohmann, P., Anten, N. P. R., & Weih, M. (2023). Editorial: Breeding for intercropping. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1143653
- Schmutz, A., Schöb, C., Rey Juan Carlos, U., & Correspondence Anja Schmutz, S. (2023). Crops grown in mixtures show niche partitioning in spatial water uptake. *Journal of Ecology*, *111*(5), 1151–1165. https://doi.org/10.1111/1365-2745.14088
- Stefan, L., Engbersen, N., & Schöb, C. (2022). Rapid transgenerational adaptation in response to intercropping reduces competition. *ELife*, *11*, 77577. https://doi.org/10.7554/ELIFE.77577
- Stomph, T. J., Dordas, C., Baranger, A., de Rijk, J., Dong, B., Evers, J., Gu, C., Li, L., Simon, J., Jensen, E. S., Wang, Q., Wang, Y., Wang, Z., Xu, H., Zhang, C., Zhang, L., Zhang, W. P., Bedoussac, L., & van der Werf, W. (2020). Designing intercrops for high yield, yield stability and efficient use of resources: Are there principles? *Advances in Agronomy*, *160*(1), 1–50. https://doi.org/10.1016/BS.AGRON.2019.10.002
- Wisheu, I. C., & Keddy, P. A. (1992). Competition and centrifugal organization of plant communities: theory and tests. *Journal of Vegetation Science*, *3*(2), 147–156. https://doi.org/10.2307/3235675
- Wolfe, M. D., Jannink, J. L., Kantar, M. B., & Santantonio, N. (2021). Multi-Species Genomics-Enabled Selection for Improving Agroecosystems Across Space and Time. *Frontiers in Plant Science*, *12*, 665349. https://doi.org/10.3389/FPLS.2021.665349/BIBTEX
- Wuest, S. E., Schulz, L., Rana, S., Frommelt, J., Ehmig, M., Pires, N. D., Grossniklaus, U., Hardtke, C. S., Hammes, U. Z., Schmid, B., & Niklaus, P. A. (2023). Single-gene resolution of diversity-driven overyielding in plant genotype mixtures. *Nature Communications 2023 14:1*, 14(1), 1–11. https://doi.org/10.1038/s41467-023-39130-z
- Zannone, L., Rotili, P., Paoletti, R., Scotti, C., Zannone, L., Rotili, P., Paoletti, R., & Scotti, C. (1986). Experimental studies of grass-legume associations. *AgSD*, *6*(10), 931–940. https://doi.org/10.1051/AGRO:19861009