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Coordinatore del Dottorato
ch. prof. Enrico Bertuzzo

Supervisore
ch. prof. Gabriella Buffa

Dottorando
Mauro Roscini
Matricola 956428

Università
Ca’Foscari
Venezia

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Introduction

Hunger and food security have been a recurring theme since the aftermath of the world wars and decolonization, and are increasingly at the center of world politics.

Indeed, in 1945, the Food and Agriculture Organization (FAO) was established, as a specialized agency of the United Nations whose main task was and is to reduce and eliminate hunger in the world (Diouf and Lowrey, 1945). To this end, the FAO, together with other United Nations agencies, established the Millennium Development Goals in 2000, the first of which was to halve the number of undernourished people suffering from hunger by 2015 (Sachs, 2012). In 2015, again in synergy with all UN agencies, the 2030 Agenda for Sustainable Development was developed, a declaration with 17 goals to be achieved by 2030, the second most important of which is to end hunger in the world, namely "End hunger, achieve food security and improved nutrition, and promote sustainable agriculture" (UN, 2015). Until 2015, the percentage of undernourished people had been decreasing for decades, but 2015 unfortunately saw a reversal of the trend and future food security is seriously threatened by several factors (FAO, 2021). The first is the constant increase in the world's population. According to recent studies, the world population will grow to about 10 billion people by 2050 (Godfray et al., 2010).

In order to reduce world hunger and increase agricultural productivity, the Green Revolution took place from the 1970s onwards, i.e., a major transformation and industrialization of the entire agricultural production system, which took place mainly in the more developed Western countries. Specifically, a massive use of external inputs, pesticides and pest control agents, the use of agricultural machinery and new technical means were introduced. However, the most important innovation was the creation and complete dissemination of highly productive and genetically and phenotypically homogeneous plant varieties to facilitate mechanized cultivation (Evenson and Gollin, 2003). The green revolution has led to a sharp increase in production. For example, rice production increased from 60 million tons in 1970 to 135 tons in 2000. In Asia, malnutrition has even declined significantly, from 47% in the 1960s to 17% in the 2000s (FAO, 2021).

However, modern agriculture nowadays is no longer sustainable across generations due to the heavy use of external inputs, namely water and chemicals (Altieri, 2009). Moreover, the agricultural sector is one of the largest sources of soil and water pollution, as well as a source of greenhouse gases from agricultural machinery and nitrogen volatilization from chemical fertilizers (Mateo Sagasta et al., 2017). Although the agricultural sector is one of the largest emitters of greenhouse gases, it is also one of the sectors most affected by pollution and climate change (Myers et al., 2017). The IPCC 2021 report (IPCC, 2021) on climate change highlights how rising temperatures will cause a change in the hydrological cycle. Precipitation will decrease but be more intense, river flow regimes will change, processes of evapotranspiration and accumulation of water and moisture in the soil as well. Extreme events such as droughts, hail, strong winds and heat waves will increase and may in turn trigger phenomena such as fires, floods, and landslides (IPCC, 2021). The impact will vary from region to region, depending on future scenarios of carbon dioxide emissions into the atmosphere.

The possible measures taken in the management of agricultural activities must take into account a number of impacts on the sector:

- Agricultural yields will fluctuate more from year to year (Gammans et al., 2017)
- Multiplication and spread of new insect and weed species is possible, with significant impacts on agricultural production (Baijwa et al., 2020)
- Management of plant pests and diseases will require adjustments in the timing, types, and effectiveness of chemical and biological control measures (Baijwa et al., 2020)

- There will be a need to deal with drier summers and higher water demand for intensive crops (Gammans et al., 2017)
- Sea level rise will lead to salinization of surface and subsurface water resources and affect water supply in coastal areas (Zalidis et al., 2002; Scotti et al., 2015).

For the Mediterranean region, it is estimated that climate change will cause a decrease of about 33% in conventional cereal production by 2050 (Gammans et al., 2017).

Climate change can affect forage quality in different ways. According to Giridhar and Samireddypalle (2015), the increase in temperature leads to an early elongation of the stem, alongside with a faster degradation of the cell wall and thus a faster decline in the digestibility of forages. Higher CO_2 concentration tends to increase total non-structural carbohydrate content by 25% and decrease N content by 8%, changing the C:N ratio of crops (Kamran et al., 2020). It is estimated that climate change will reduce feed grain yields in the Mediterranean region by 20-36%, resulting in huge economic losses estimated at €36 billion for the agricultural sector (Shrivastava and Kumar, 2016).

The main reason for this strong vulnerability of the modern agricultural sector to climate change is its extreme homogenization and industrialization in all production sectors (Altieri, 2009). In particular, genetically and phenotypically homogeneous varieties are extremely vulnerable to all climatic changes and all resulting stress factors. Moreover, the genetic creation of varieties that are not susceptible to herbicides will accelerate the development of increasingly harmful and pesticide-resistant superweeds (Altieri, 2009). This is precisely why recent studies indicate that the most important way to mitigate and adapt to climate change is to promote and enhance agrobiodiversity (Kahane et al., 2013). Agrobiodiversity contributes to the procurement, regulation, and support of ecosystem services and in many developing countries smallholder farmers use diversity as an integral part of their livelihood strategies (Kahane et al., 2013). Jarvis et al. (2011) proved that the use of crop diversity enables adaptation to marginal ecosystems and heterogeneous environments, hedging against environmental and other risks, optimal management of pests and diseases and, most importantly, yield stability. To this end, the reintroduction of traditional cereal varieties characterized by high genetic diversity, strong resistance to pathogens and lower demand for nutrients and agricultural practices is being explored (Maroni and Ponzini, 2018). Traditional varieties were created for generational selection, i.e., the systematic annual selection of the best plants best suited to the soil and local climate (Ceccarelli, 2016). In Italy, traditional varieties strongly developed in the first post-war period, in the so-called "Battaglia del Grano", which aimed to increase production so that the entire national demand for wheat was covered by the domestic market alone. However, with the advent of the Green Revolution and industrial agriculture in 1950, traditional varieties were replaced by modern varieties, which were characterized by a much higher yield than the traditional cereals and which could be subjected to industrial processing without being damaged (Maroni and Ponzini, 2018). Traditional varieties are on average taller and more heterogeneous compared to modern cereals, which makes them less suitable for the massive mechanization of agricultural practices. However, they are more competitive against weeds thereby requiring less or no use of environmentally harmful herbicides and fertilizers (Maroni and Ponzini, 2018). In recent years, there has been a slow return to the use of traditional varieties, as they have a diverse genetic heritage that can evolve and thus better adapt to the environment in which they occur without requiring a high energy input. Given these characteristics, it is worth studying their potential tolerance and adaptability to new climatic conditions and the resulting stresses. If confirmed, traditional varieties could ensure good productivity even under limiting conditions (Moudry et al., 2012), and offer the possibility to use currently marginal land, thereby ensuring profit and economic livelihood (Ceccarelli, 2016).

For this reason, the overall aim of this project was to assess the tolerance of some traditional cereal varieties most commonly used in Mediterranean regions to climatic stress factors (high temperatures, drought, salinity) and to characterize them from a morpho-agronomic point of view (germination, growth, biomass parameters) in order to identify those varieties that could be used to cope climate change and to adapt the agricultural sector to climate change.

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Response of the Traditional Varieties to heat, drought, salt stress and their interaction

Abstract

Climate change is one of the biggest threats to food security, causing extreme weather phenomena such as severe temperature fluctuations, extreme precipitation, floods, droughts and salinization, that are also expected to become more frequent. This exposes crop species to increased abiotic stress thereby affecting production. The objective of this part of the project was to investigate tolerance to salt, drought and heat stress and their interaction at the germination stage in traditional cereal cultivars. For this research the following traditional varieties have been selected and tested, i.e., "Einkorn" *Triticum monococcum* L, "Emmer" *T. dicoccum* L., *T. durum* Desf. var. Senatore Cappelli, *T. aestivum* L. var. Verna, *T. durum* Desf. var. Timilia, *T. aestivum* L. var. Gentil Rosso, *T. turanicum* Jakubz., and one modern variety, the *T. durum* Desf. var. Claudio. The research has shown that all three abiotic stress factors significantly affect seed germination and viability, moreover traditional varieties showed their greater tolerance to all the three stress factors investigated, in comparison with the modern variety (Claudio).

Introduction

The IPCC's 6th Assessment Report contains new confirmation of the anthropogenic origin of climate change. The IPCC models indicate a robust and statistically significant increase of 1 to 5.5 °C according to the different representative concentration pathways (IPCC, 2014). Furthermore, due to deforestation and overuse of fossil fuels, atmospheric CO_2 levels have increased from 280 to 400 ppm and are projected to increase to 800 ppm by 2100 (IPCC, 2021). Climate change is one of the biggest threats to food security, causing extreme weather phenomena such as severe temperature fluctuations, extreme precipitation, floods, droughts and salinization, that are also expected to become more frequent. This exposes crop species to increased abiotic stress thereby affecting production.

The Food and Agriculture Organization of the United Nations (FAO) has calculated that by 2020, global productivity loss will affect 46 million people per year causing hunger and malnutrition (FAO 2020), and with an ever-growing population, this trend will only worsen unless innovative and structural measures are taken to combat world hunger (FAO, 2020).

Prolonged or sporadic heat stress could drastically alter crop distribution range and shorten the growing season, leading to a decline in growth and yields of all major crops. High temperatures affect crops in a number of ways, including poor plant growth, reduced photosynthesis, leaf senescence, reduced pollen viability and consequently the production of fewer grains with smaller grain size (McClung and Davis 2010; Grant et al. 2011). Closely related to heat stress are drought and salt stress. Soil salinity already affects 20% of total cropland and 33% of irrigated agricultural land worldwide (Srivastava and Kumar, 2015) and is projected to increase even faster than today by 2050 (Singh et al., 2021). Salt stress negatively affects yield by 10 to 50% as most plant species are sensitive to salt concentration of 1.0-1.8 dS m-1. Salt affects various plant processes and leads to nutritional imbalance, ion toxic effects and oxidative stress (Almansouri et al., 2001), and has greater adverse effects on plants than osmotic stress due to the toxic effect of ions. Drought stress affects 45% of arable land and the global area suffering from drought stress has increased from 17 to 27% between 1950 and 2020 (Kapoor et al., 2020). Drought stress can lead to changes in gene expression that are important for plant life support (Batlang et al. 2013), reduced protein production that can damage crops (Mohammadi et al. 2012), and changes in metabolic activity that affect crop production (Kumari et al. 2016). In recent decades, yield losses of about 21% in wheat and 40% in maize have been observed due to drought (Zhang et al., 2018).

In the scenario outlined above, agriculture plays a dual role, being one of the most important sources of greenhouse gases and climate change, and also one of the most affected production sectors. The current agricultural system is not able to cope with the challenges of climate change because it is based on intensive production methods and the overuse of genetically homogeneous, high-yielding wheat varieties (Kahane et al., 2013). Indeed, low genetic diversity is associated with lower stress resistance and resilience. To adapt agriculture to climate change, the introduction and improvement of genetic and agrobiodiversity in the system is essential (Kahane et al., 2013).

Italian traditional cereal varieties have greater genetic diversity compared to modern varieties, due to adaptation to specific climatic and edaphic conditions in the XX century (Casañas et al., 2017). Furthermore, Piergiovanni (2013) reported that consumer demand for traditional varieties is increasing as consumers see the benefits of freshness, better taste and higher quality.

However, agronomic and physiological studies show that plant response to concomitant abiotic stresses cannot be extrapolated directly from the application of each stress. Since germination evaluation is considered a rapid and efficient method to assess cultivar stress tolerance (Ashraf et al., 1987), our objective was to investigate tolerance to salt, drought and heat stress and their interaction at the germination stage in traditional cereal cultivars, including a wide range of salt and drought stress levels and extending the temperature range expected in Veneto under climate change scenarios.

Methods

Seed collection

For this research the following traditional varieties have been selected and tested, i.e., "Einkorn" *Triticum monococcum* L, "Emmer" *T. dicoccum* L., *T. durum* Desf. var. Senatore Cappelli, *T. aestivum* L. var. Verna, *T. durum* Desf. var. Timilia, *T. aestivum* L. var. Gentil Rosso, *T. turanicum* Jakubz., and one modern variety, the *T. durum* Desf. var. Claudio. Seeds were obtained from the

Rural Seed Network of Umbria and Los Prados Company. The chosen eight cereal varieties are briefly descripted in Table 1. The tested varieties were stored at 18 °C and 40% humidity in the storage cell of Ca' Foscari University.

Table 1 Description of the tested varieties (De Cillis, 1927; Ghiselli et al., 2016; Trebbi et al., 2011)

Variety	Species	Registration Year	Country	Pedigree
Verna	*Triticum aestivum* L.	1953	Italy	Est Mottin/Mont Calme 245
Einkorn	*Triticum monococcum L.*	/	Italy	/
Emmer	*Triticum dicoccum L.*	/	Italy	/
Senatore Cappelli	*Triticum durum Desf.*	1930	Italy	Selection from Jennah Rhetifa
Claudio	*Triticum durum Desf.*	1999	Italy	CIMMYT35/Durango//IS1938/Grazia
Timilia	*Triticum durum Desf.*	/	Italy	/
Turanico	*Triticum turanicum Jakubz.*	1921	Italy	/
Gentil Rosso	*Triticum aestivum L.*	1850	Italy	/

Germination tests

The quality and viability of each seed lot was estimated by the cut test (Baskin and Baskin, 2014). Accordingly, only seeds with turgid, healthy tissue without damage from pathogens or insects were considered potentially viable (Davies et al., 2015).

Seed viability was calculated as the percentage of potentially viable seeds averaged over 4 replicates for each lot. Each seed lot was characterised by high quality seeds with a percentage of potentially viable seeds greater than 90%. To avoid microbiological infestation, seeds were sterilised in a 1% sodium hypochlorite solution for 1 minute. A 2-factor experiment was carried out. For each factor combination, 10 replicates of 20 seeds each were sown in a sterile environment on two layers of No. 1 Whatman paper in 9 cm diameter Petri dishes.

To assess sensitivity to heat, salt stress and their interaction, seeds were exposed to increasing temperatures (20°C, 25°C, 30°C ± 1.5°C) and NaCl concentrations (0 mM, 50 mM, 100 mM, 150 mM, 200 mM, 300 mM. To assess drought stress tolerance and interaction with high temperatures, seeds were tested with different mannitol concentrations to expose the cultivars to different osmotic potential, i.e., 0, -0.25 Mpa, -0.5 MPa, -0.75 MPa, - 1 Mpa, - 1.25 Mpa, - 1.5 MPa, and subjected to the same thermal regimes as in the salt stress test. These osmotic potentials were chosen because they correspond to those caused by the NaCl concentrations in order to illustrate the toxic effect of ions during salt stress.

Seeds were incubated in a growth chamber for 14 days. Germination was evaluated every 24 hours throughout the test period. Evaluation was carried out in a laminar flow bonnet to minimise microbiological contamination (Baskin and Baskin, 2014). At the end of each test, the viability of the non-germinated seeds was assessed using the cutting test. This revealed that all seeds were dead as they collapsed when lightly pinched.

Statistical Analysis

To analyse the germination response of the seven varieties under increasing heat, salt and drought stress and their interactions, we calculated three parameters, averaged by the 10 replicates for each tested condition: a) germination percentage, calculated as the number of germinated seeds on the total number of sown seeds, multiplied by one hundred; b) median germination time (T50), namely the time (in days) required to achieve 50% of germination; c) germination delay, as the number of days until the first germination occurred (Baskin and Baskin, 2014).

For each cultivar, germination percentage, T50 and germination delay were compared by a two-way PERMANOVA test with 999 randomisations, using NaCl concentration, Osmotic Potential or Temperature and their interaction as grouping variables. Fisher's least significant difference (LSD) was then performed to detect significant differences in calculated parameters between different stress concentrations.

Results

Heat stress experiments

The germination tests in optimal osmotic conditions performed at 20°C, 25°C and 30°C allowed to evaluate heat tolerance of varieties (Table 2, Table 3, Table 4, Table 5). Senatore Cappelli showed an outstanding heat tolerance, with no significant decreases in the germination in the 3 tested thermal regimes (LSD Test $p<0.005$; Table 3). Einkorn and Timilia showed a low decrease (2.5%) of seed germination, while Claudio exhibited the greatest vulnerability to heat stress, with a reduction of 15%.

Table 2 PERMANOVA result of the germination percentage, median germination time and germination delay among the tested varieties and different temperature

	Germination Percentage			Median Germination Time			Germination Delay		
	DF	F Model	P-value	DF	F Model	P-value	DF	F Model	P-value
Variety	7	15.886	0.001	7	60.81	0.001	7	32.95	0.001
Temperature	2	143.827	0.001	2	577.88	0.001	2	784.61	0.001
Variety x Temperature	4	18.891	0.001	4	25.58	0.001	4	23.96	0.001
Residuals	56			56			56		

Table 3 Means ± SD for germination percentages, of the tested varieties. Values followed by different capital letters significantly differ across varieties at the same Temperature (Fisher LSD test).

Values followed by small letters significantly differ across temperatures within each variety (Fisher LSD test).

	20 °C	25 °C	30 °C
Claudio	92.25±4.15 Aa	83.75±3.76 Ab	85.25±3.83 Ab
Verna	98±4.41 Ba	95.5±4.29 Bb	94.75±4.26 Bb
Senatore Cappelli	98.75±4.44 Ba	98.5±4.43 Ca	98.25±4.42 Aa
Einkorn	99±4.455 Ba	95.75±4.30 Bb	95±4.27 Bb
Emmer	94.75±4.26 Ba	92.25±4.15 Db	91±4.09 Cb
Timilia	98.75±4.44 Ba	96.875±4.35 Bb	95±4.27 Bb
Gentil Rosso	95.5±4.29 Ba	90.1±4.05 Db	89.5±4.027 Ab
Turanico	97.5±4.38 Ba	95.25±4.28 Bb	94±4.23 Bb

Table 4 Means ± SD for germination delay, of the tested varieties. Values followed by different capital letters significantly differ across varieties at the same Temperature (Fisher LSD test). Values followed by small letters significantly differ across temperatures within each variety (Fisher LSD test).

	20 °C	25 °C	30 °C
Claudio	0.69± 0.03 Aa	0.92± 0.05 Ab	0.97± 0.04 Ac
Verna	0.53± 0.03 Ba	0.6± 0.03 Bb	0.62± 0.03 Bb
Senatore Cappelli	0.51± 0.03 Ba	0.52± 0.03 Ca	0.52± 0.03 Ca
Einkorn	0.5± 0.03 Ba	0.59± 0.03 Bb	0.61± 0.03 Bb
Emmer	0.62± 0.03 Ca	0.69± 0.03 Bb	0.72± 0.04 Db
Timilia	0.51± 0.03 Aa	0.56± 0.03 Cb	0.61± 0.03 Bc
Gentil Rosso	0.6± 0.03 Ca	0.74± 0.04 Bb	0.76± 0.04 Db
Turanico	0.54± 0.03 Aa	0.6± 0.03 Bb	0.64± 0.03 Bc

Table 5 Means ± SD for T50, of the tested varieties. Values followed by different capital letters significantly differ across varieties at the same Temperature (Fisher LSD test). Values followed by small letters significantly differ across Temperature within each variety (Fisher LSD test).

	20 °C	25 °C	30 °C
Claudio	1.00 ± 0 Aa	1.30 ± 0.20 Ab	2.48 ± 0.40 Ab
Verna	0.65 ± 0.09 Ba	0.73 ± 0.45 Ba	0.87 ± 0.37 Bb
Senatore Cappelli	0.50 ± 0.03 Ba	0.50 ± 0.01 Ba	0.51 ± 0.07 Bb
Einkorn	0.50 ± 0.05 Ba	0.58 ± 0.13 Ba	0.73 ± 0.15 Bb
Emmer	0.60 ± 0.04 Ba	0.63 ± 0.31 Bb	1.20 ± 0.44 Ac
Timilia	0.50 ± 0.03 Ba	0.50 ± 0.01 Ba	0.51 ± 0.07 Bb
Gentil Rosso	0.65± 0.03Ba	0.74± 0.04Ba	0.86± 0.04Bb
Turanico	0.57± 0.03Ba	0.6± 0.03Ba	0.84± 0.03Bb

Drought stress experiments

At optimal thermal conditions (20°C), high levels of drought negatively affect all the varieties tested (Figure 1), leading to a significant reduction in the percentage of germination (PERMANOVA test; $p < 0.05$; Table 2 and Table 4). As observed in germination at 20°C and 25°C, the modern variety Claudio was most susceptible to drought stress, and at -1.5 Mpa germination declined by 50%. In contrast, greater tolerance was observed in the traditional varieties Verna, Monococco, Timilia and Turanico (PERMANOVA test; $p < 0.05$; Table 6 and Table 7), with an average decline in germination percentage of only 20%. The drought stress test conducted at 25 and 30°C showed a significant influence of temperature on germination (PERMANOVA test, $p < 0.05$; Table 7). Moreover, the greater tolerance of the traditional varieties was also confirmed at 25°c and 30°C with an average germination of 73%. In contrast, Claudio and Senatore Cappelli showed a drastic decrease to 43.3% and 30.3%, respectively, at 25°C and an osmotic potential of -1.5 MPa and to 36.6% and 24%, respectively, at 30°C with the same osmotic potential. The analysis of germination delay and T50 confirmed the trends observed in germination percentage analysis. In fact, a negative influence of drought stress and temperature on the varieties was also observed for these two parameters (PERMANOVA test, $p < 0.05$; Table 7); however also in this case the traditional varieties showed a greater tolerance (Figure 3). Indeed, the modern variety Claudio showed the longest germination delay (2.5 days) at 30°C and 300 mM of NaCl, while the variety Senatore Cappelli had the longest T50 value (5.50 days) (Figure 2).

Table 6 PERMANOVA result of the germination percentage, median germination time and germination delay among the tested varieties and different Osmotic Potential, temperature and their interaction.

	Germination Percentage			Median Germination Time			Germination Delay		
	DF	F Model	P-value	DF	F Model	P-value	DF	F Model	P-value
Variety	7	45.45	0.001	7	173.92	0.001	7	91.52	0.001
Osmotic Potential	6	103.00	0.001	6	415.28	0.001	6	568.72	0.001
Temperature	2	373.27	0.001	2	655.90	0.001	2	636.14	0.001
Variety x Osmotic P.	42	10.52	0.001	42	13.80	0.001	42	13.23	0.001
Temperature x Osmotic P.	14	28.22	0.001	14	38.80	0.001	14	37.19	0.001
Residuals	448			448			448		

Table 7 PERMANOVA result of the germination percentage, median germination time and germination delay among the tested varieties and different Osmotic Potential.

		Germination Percentage			Median Germination Time (T50)			Germination Delay		
		DF	F Model	P-value	DF	F Model	P-value	DF	F Model	P-value
Claudio	Osmotic P	1	11.956	0.001	1	60.976	0.001	1	65.732	0.001
	Temperature	1	50.599	0.001	1	258.404	0.001	1	278.705	0.001
	Osmotic P x Temperature	1	11.694	0.001	1	59.639	0.001	1	64.291	0.001
Verna	Osmotic P	1	14.852	0.001	1	22.876	0.001	1	25.433	0.001
	Temperature	1	62.857	0.001	1	96.945	0.001	1	107.836	0.001
	Osmotic P x Temperature	1	14.527	0.001	1	22.375	0.001	1	24.888	0.001
Einkorn	Osmotic P	1	10.028	0.001	1	3.904	0.001	1	105.195	0.001
	Temperature	1	42.441	0.001	1	16.543	0.001	1	446.03	0.001
	Osmotic P x Temperature	1	9.809	0.001	1	3.818	0.001	1	102.943	0.001
Emmer	Osmotic P	1	18.844	0.001	1	23.837	0.001	1	126.732	0.001
	Temperature	1	64.198	0.001	1	101.015	0.001	1	537.35	0.001

	Osmotic P x Temperature	1	18.431	0.001	1	23.314	0.001	1	124.019	0.001
Senatore Cappelli	Osmotic P	1	20.02	0.001	1	140.91	0.001	1	140.836	0.001
	Temperature	1	101.015	0.001	1	597.15	0.001	1	597.15	0.001
	Osmotic P x Temperature	1	19.582	0.001	1	137.822	0.001	1	137.821	0.001
Timilia	Osmotic P	1	9.928	0.001	1	3.826	0.001	1	103.091	0.001
	Temperature	1	42.017	0.001	1	16.212	0.001	1	437.109	0.001
	Osmotic P x Temperature	1	9.711	0.001	1	3.742	0.001	1	100.884	0.001
Turanico	Osmotic P	1	24.373	0.001	1	22.419	0.001	1	24.924	0.001
	Temperature	1	61.6	0.001	1	95.006	0.001	1	105.679	0.001
	Osmotic P x Temperature	1	23.839	0.001	1	21.927	0.001	1	24.391	0.001
Gentil Rosso	Osmotic P	1	12.344	0.001	1	23.36	0.001	1	124.198	0.001
	Temperature	1	62.914	0.001	1	98.995	0.001	1	526.603	0.001
	Osmotic P x Temperature	1	12.074	0.001	1	22.848	0.001	1	121.539	0.001
	Residuals	56			56			56		

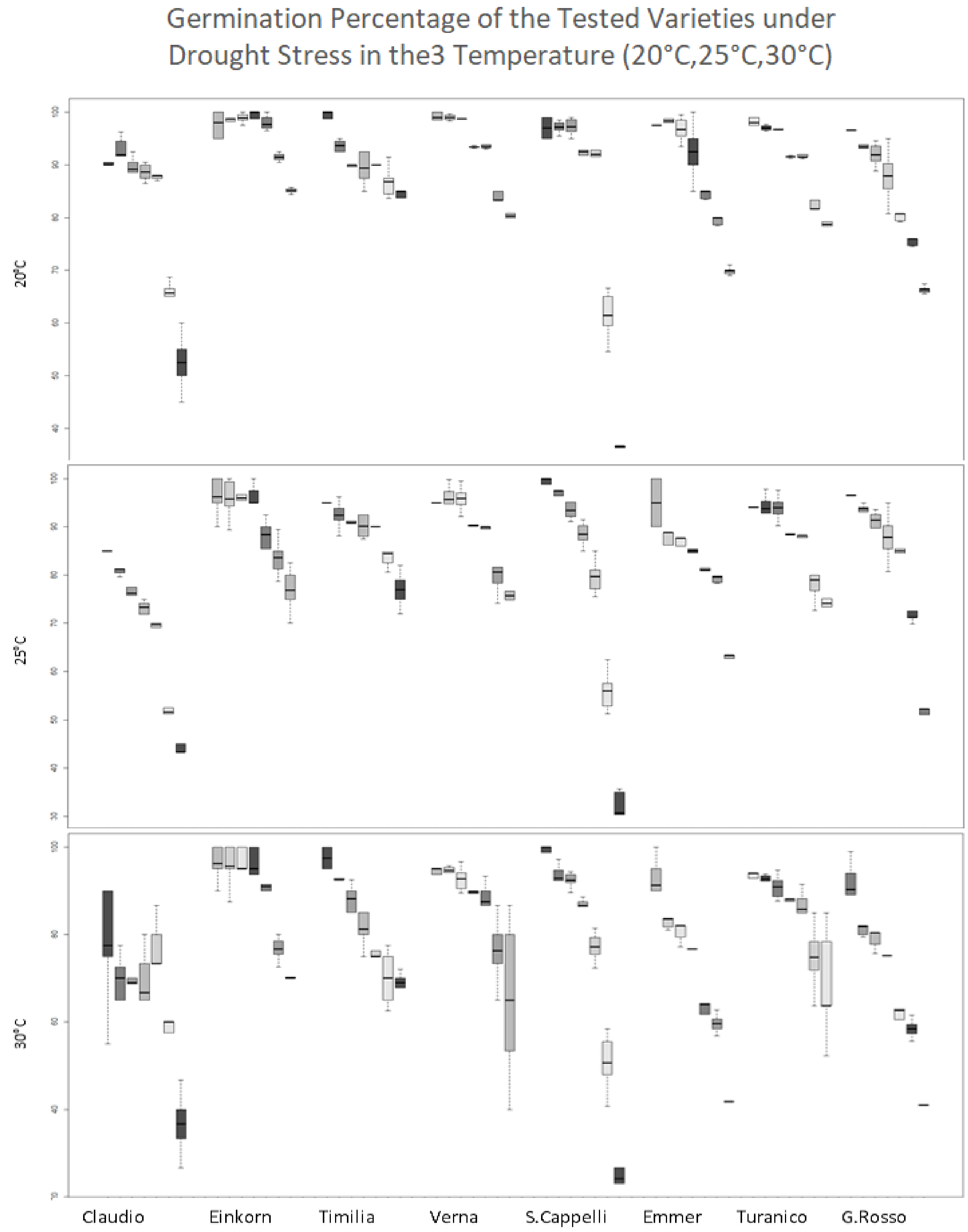
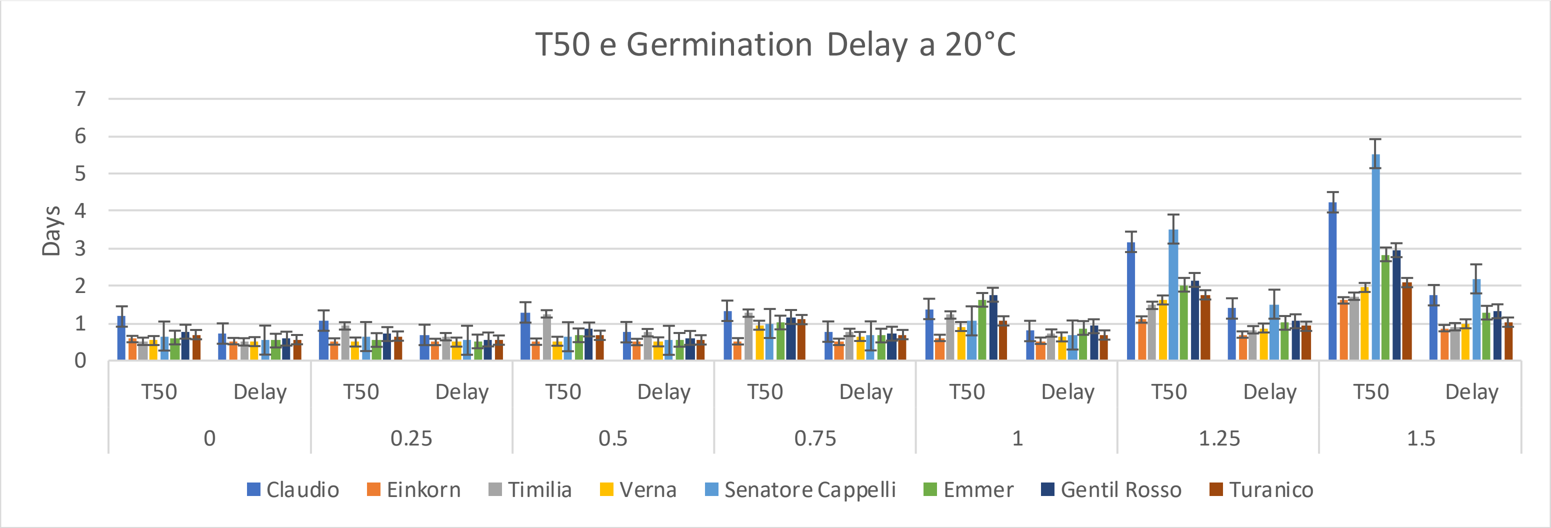
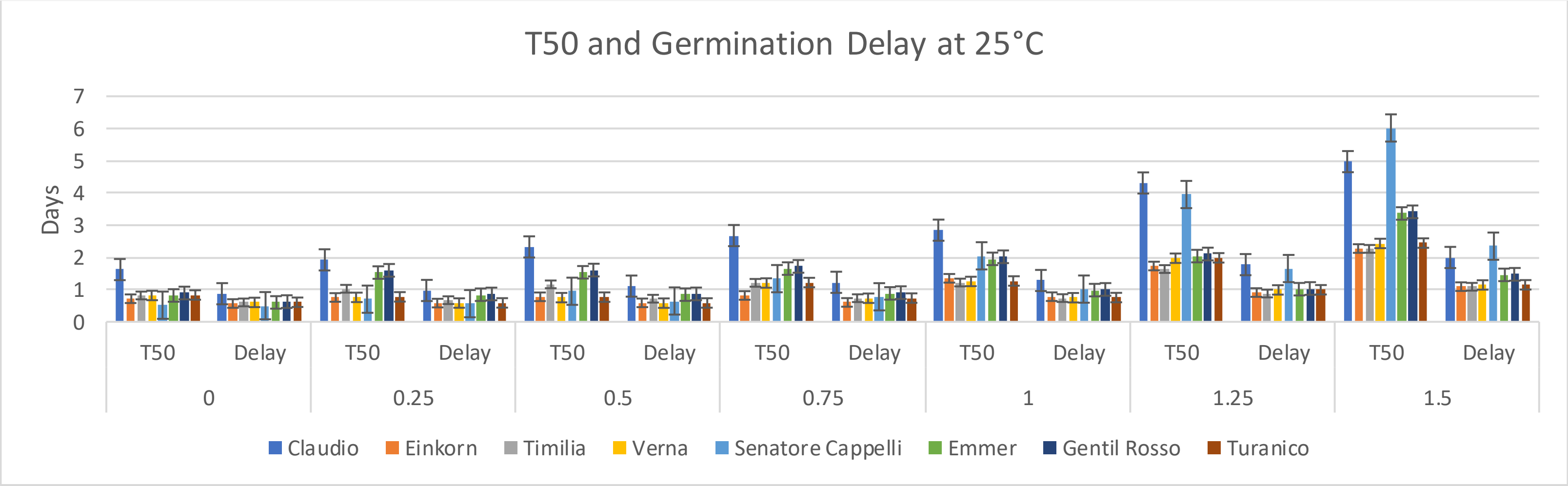


Figure 1 Germination percentage of the tested varieties under Osmotic stress in 3 Temperature (20°C,25°C,30°C)


T50 e Germination Delay a 20°C


T50 and Germination Delay at 25°C

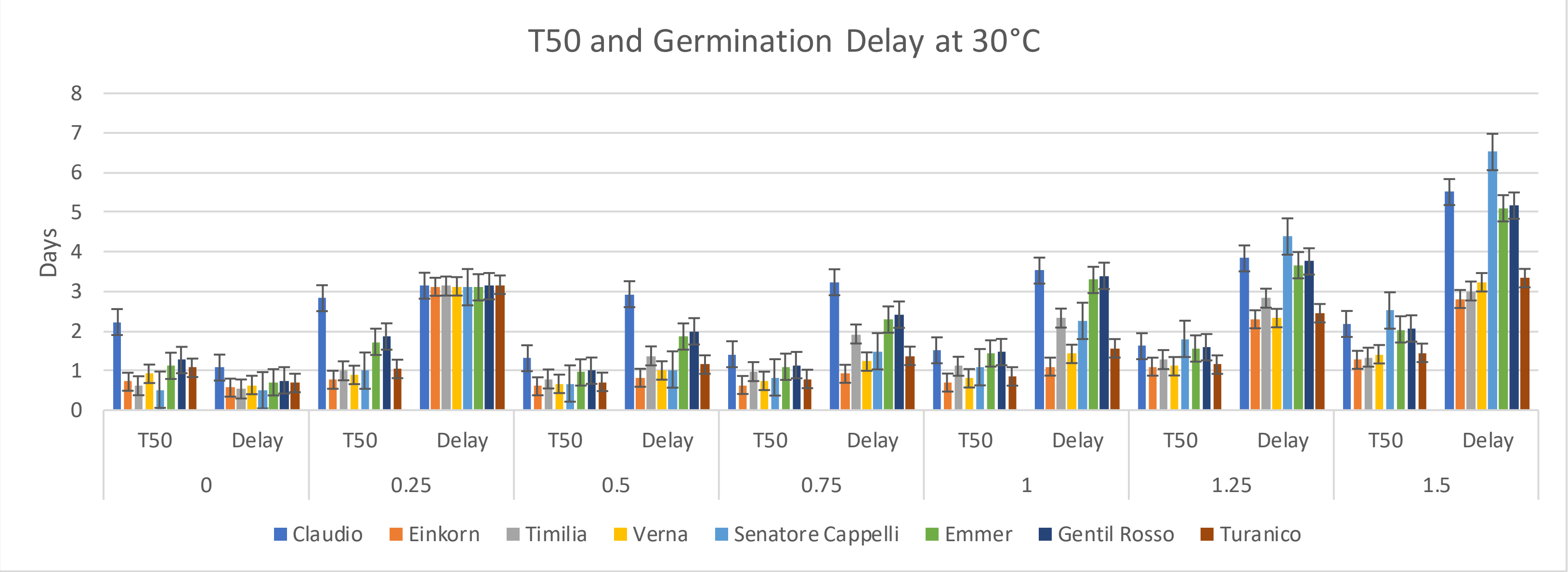


Figure 2 T50 and Germination Delay under Drought Stress in 3 thermal regimes (20 °C, 25°C, 30°C)

Salt stress experiments

Under optimal thermal conditions (20 °C) all the tested varieties were significantly and negatively affected by salt stress (PERMANOVA test; $p < 0.05$; Table 3), because each germination parameters have decreased (germination percentage, T50 and germination delay). However, predictably the tested cultivars showed different behaviour and tolerance to NaCl presence. In all varieties the maximum germination (96,35 ± 5,17 %, averaged across all the tested varieties) was observed in absence of salt stress (Figure 3). The traditional varieties Verna, Einkorn, Timilia and Emmer showed a greater seed vitality and germination compared to Senatore Cappelli and Claudio. Despite their germination percentage linearly decreased across increasing salt concentration, they maintained germination percentages higher than 75 % also at the highest tested concentration of NaCl (NaCl 300 mM). Conversely, the modern variety Claudio and the traditional crop Senatore Cappelli exhibited a sharper decrease in the percentage of germination at increasing salt concentration, and at NaCl 300 mM their germination percentage considerably decreased to 43% and 18.5 % respectively.

The synergistic interaction between salt and high temperatures caused a reduction in seed germination (PERMANOVA test; $p < 0.05$; Table 8). In fact, the average germination of traditional varieties at 300 mM of NaCl decreased to 65.5%, and 45.9% respectively at 25 ° C and 30 °. The harmful effect between salt and temperature was particularly evident in the modern variety Claudio and in Senatore Cappelli, indeed their germination at 25 ° C stood at 43.3% and 25% respectively, down to zero in Claudio at 30 ° C and 300 mM. The negative synergistic effect between salt and high temperatures was also evident in the evaluation of T50 and the germination delay; the modern variety Claudio showed the longest T50 (6 days) at 30°C and 300 mM of NaCl, while the variety Senatore Cappelli had the longest Germination delay (3.51 days) (Figure 4).

Drought and Salt stress tests were performed with the same osmotic potential to identify the toxic effect of salt ions by the difference between the two types of stress (Figure 5). A direct correlation was found between the toxic effect of the ions and temperature. High temperatures resulted in greater toxic exposure to the ions. The Claudio variety was the most susceptible, with about 30% of the non-germinated seeds affected by oxidative stress at 30°C. The traditional varieties are also susceptible to the harmful effects of ions, but to a lesser extent. In particular, for Verna, Einkorn and Timilia, only 5% of the non-germinated grains are affected by oxidative stress.

Table 8 PERMANOVA result of the germination percentage, median germination time and germination delay among the tested varieties and different Salt Concentratrion, temperature and their interaction.

	Germination Percentage			Median Germination Time			Germination Delay		
	DF	F Model	P-value	DF	F Model	P-value	DF	F Model	P-value
Variety	7	45.13	0.001	7	172.70	0.001	7	90.88	0.001
NaCl Concentration	6	119.27	0.001	6	480.88	0.001	6	658.56	0.001
Temperature	2	371.97	0.001	2	653.62	0.001	2	633.93	0.001
Variety x NaCl	42	10.58	0.001	42	13.88	0.001	42	13.309	0.001
Temperature x NaCl	14	27.25	0.001	14	37.47	0.001	14	35.92	0.001
Residuals	448			448			448		

Germination Percentage of the Tested Varieties under Salt Stress in the 3 Temperature (20°C,25°C,30°C)

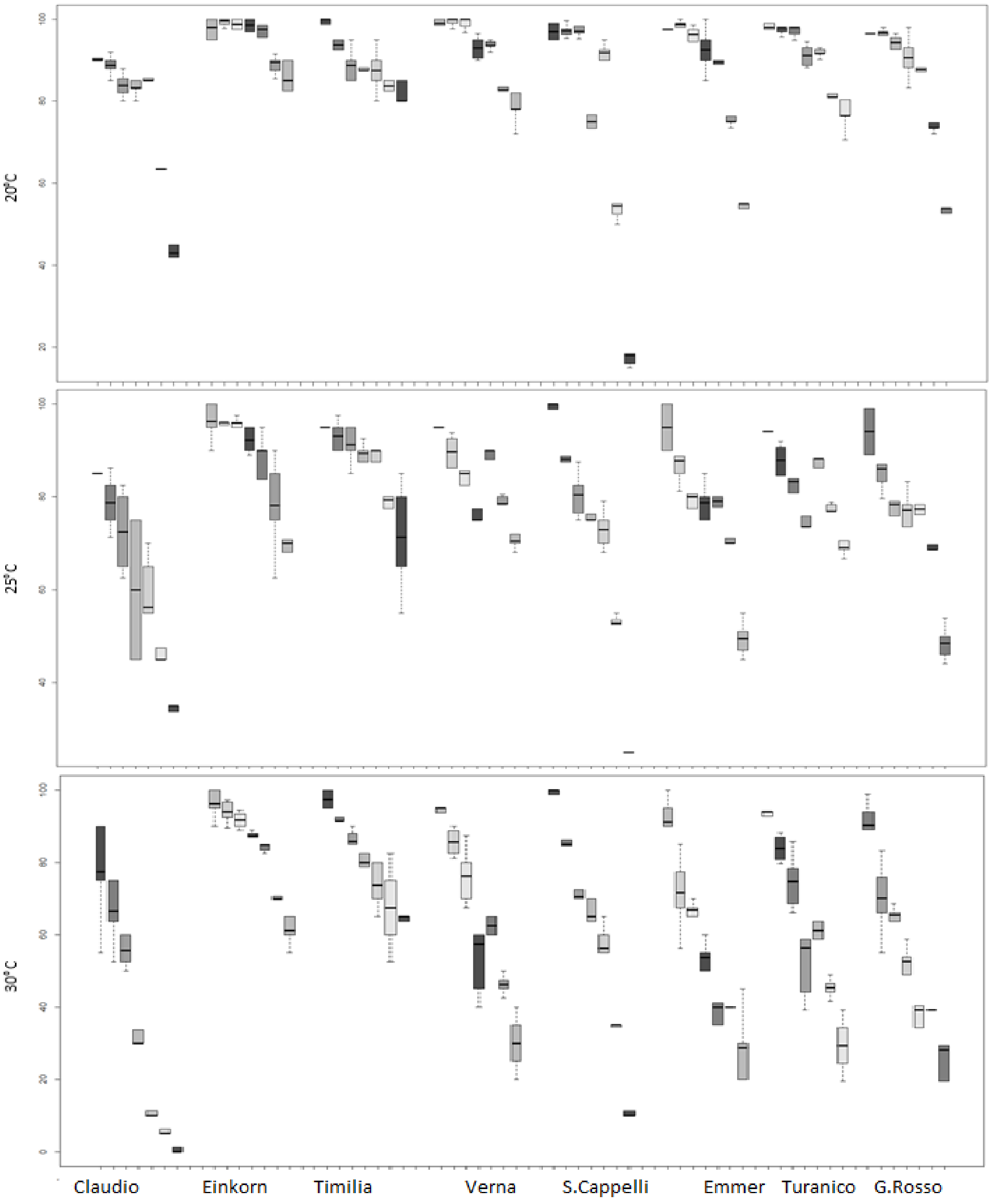
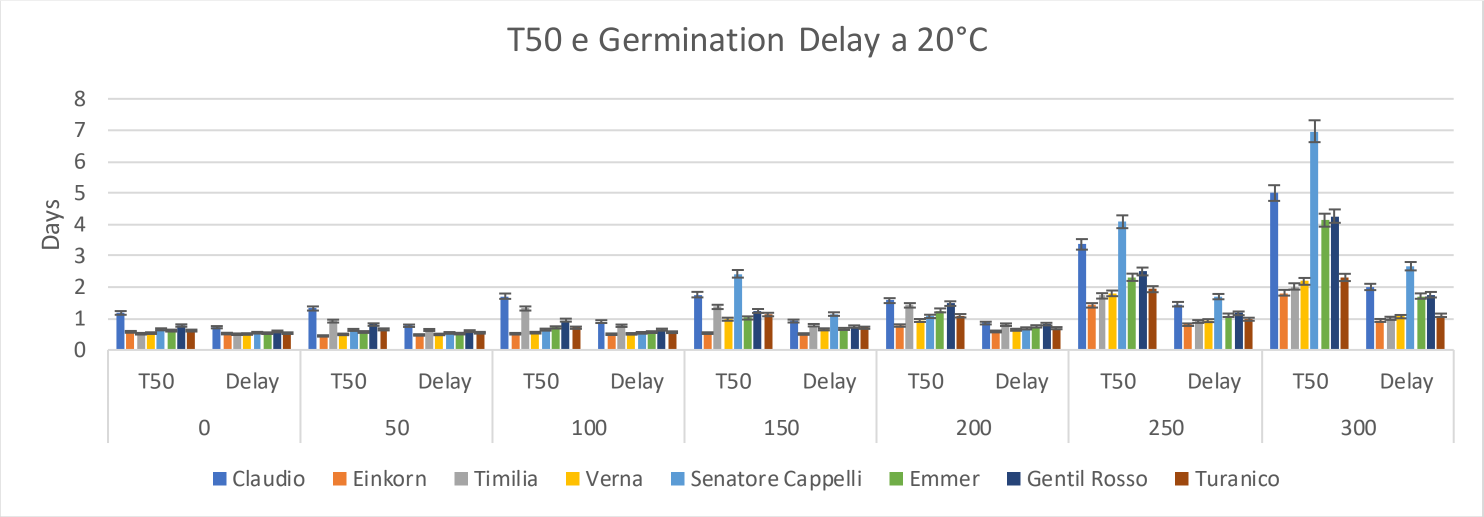
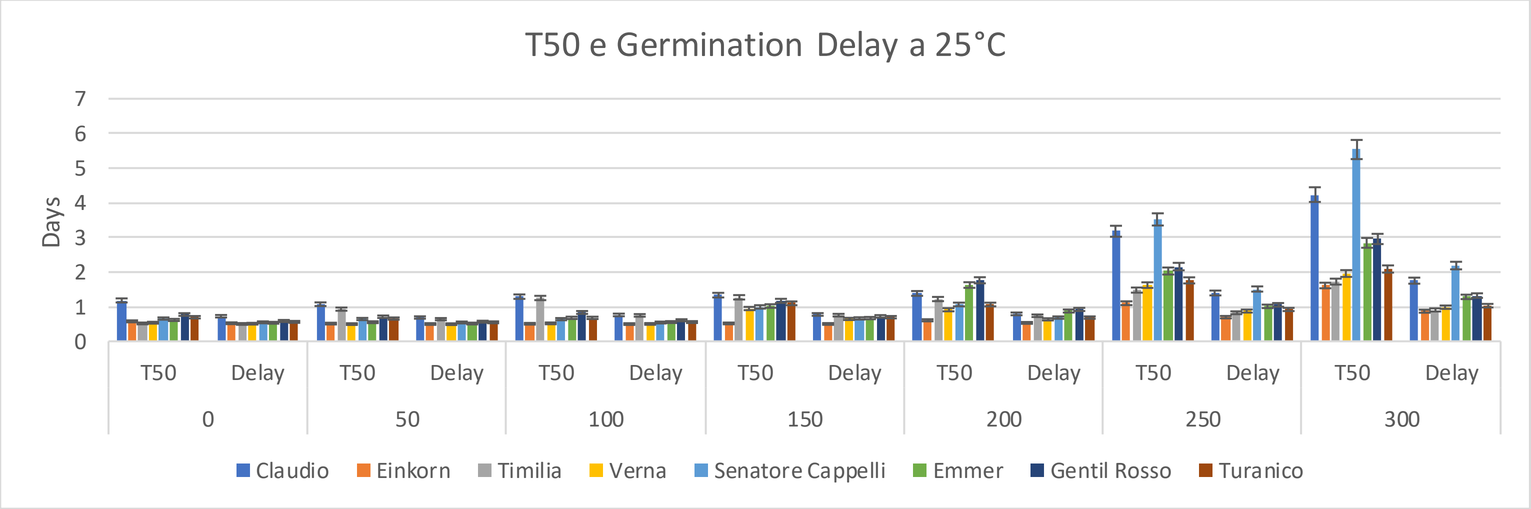


Figure 3 Germination percentage of the tested varieties under Salt stress in 3 Temperature (20°C,25°C,30°C)


T50 e Germination Delay a 20°C
Days
0
1
2
3
4
5
6
7
8
T50
Delay
0
50
100
150
200
250
300
Claudio
Einkorn
Timilia
Verna
Senatore Cappelli
Emmer
Gentil Rosso
Turanico


T50 e Germination Delay a 25°C
Days
0
1
2
3
4
5
6
7
T50
Delay
0
50
100
150
200
250
300
Claudio
Einkorn
Timilia
Verna
Senatore Cappelli
Emmer
Gentil Rosso
Turanico

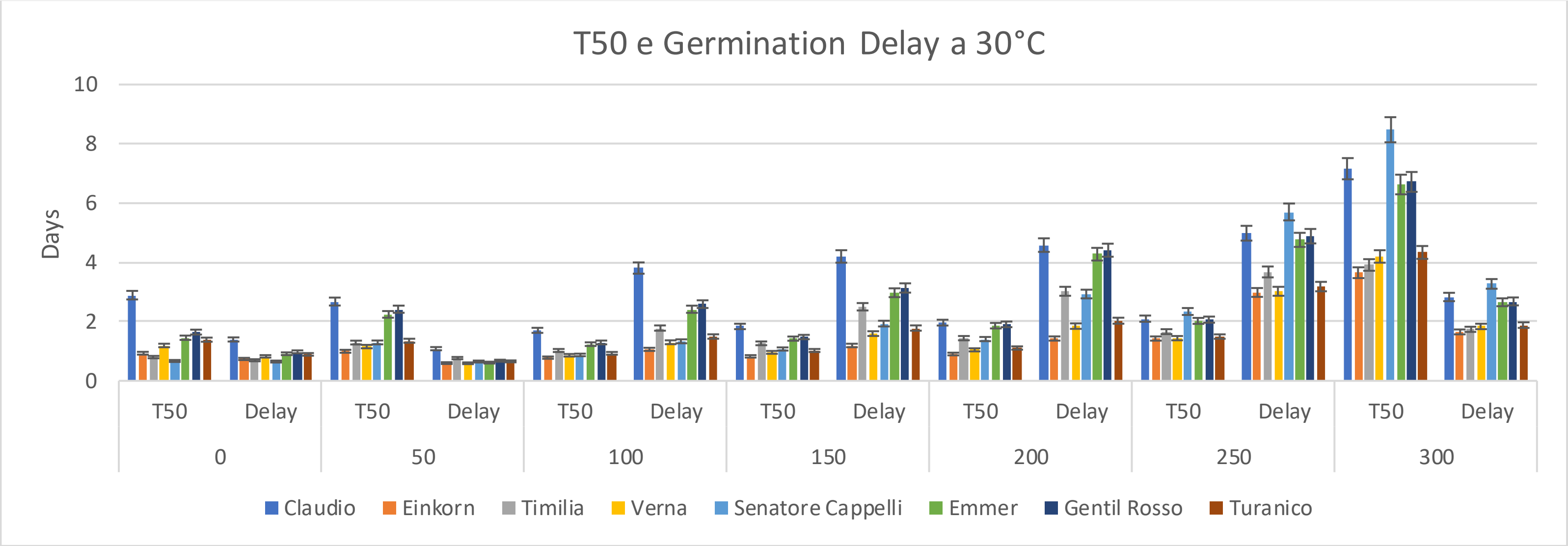


Figure 4 T50 and Germination Delay under Salt Stress in 3 thermal regimes (20 °C, 25°C, 30°C)

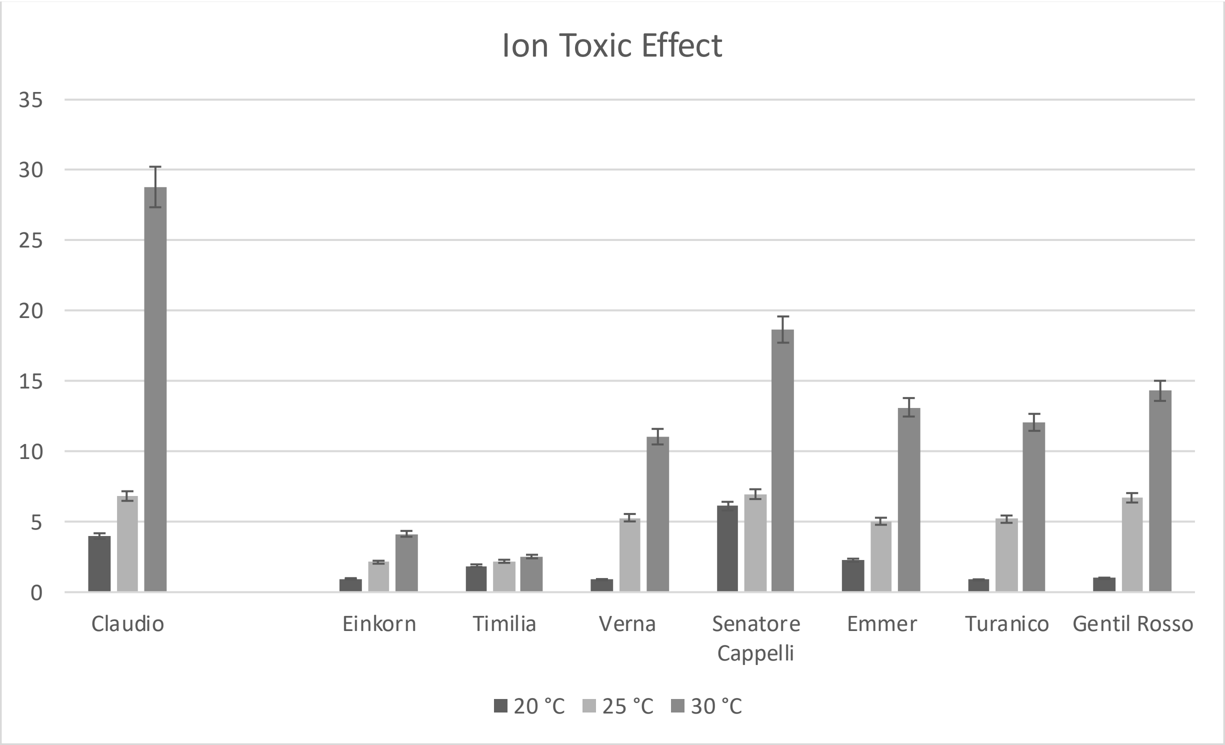


Figure 5 Ion Toxic Effect on the Tested Varieties among the 3 Thermal Regimes

Discussion

Our research has shown that all three abiotic stress factors significantly affect seed germination and viability. However, the effects of these three stress factors on germination are very different. In fact, among the 3 stress factors tested, increasing temperature leads to the lowest reduction in germination, followed by drought stress and then the most damaging stress factor, salt stress.

Under optimal osmotic conditions, high temperatures cause a greater affinity of oxygen for Rubisk enzyme at the chloroplast level, leading to a major respiration rate and a minor photosynthesis activity, as well as a slowdown in primary production and biomass accumulation (Suter and Widmer, 2013). Conversely, drought and salt stress cause a greater shock at the physiological level because the negative osmotic potentials lead to a decrease in the water available to the plant cells. Water deficiency leads to a reduction in the growth, elongation and expansion of the cell, and disturbs the antioxidant function of the plant; furthermore, salt stress has another damaging effect due to the toxic effect of the ions causing the accumulation of ROS (Reactive Oxygen Species) and the resulting oxidative damage (Osakabe et al., 2014). The harmful effect of salt stress and drought is accelerated and aggravated by high temperatures, because heat stress leads to a greater opening of the stomata with a further loss of water through transpiration and, above all, to a greater uptake of salt into the upper parts of the plants (Suzuki et al., 2016).

Nevertheless, we observed a considerable variability in germination in the varieties tested under the different stress conditions, and, according to our results, traditional varieties confirmed their greater tolerance to all the three stress factors investigated, in comparison with the modern variety (Claudio). However, a certain difference in tolerance to abiotic stress factors was found also in the traditional varieties; in fact, Senatore Cappelli showed a remarkable tolerance to heat stress, probably deriving this trait from his Tunisian ancestor *Jennah rhetifah* (De Cillis, 1927). However, Senatore Cappelli proved to be very susceptible to drought and salt stress, as its germination capacity is comparable to that of the modern variety. In contrast, Verna, Timilia and Einkorn showed remarkable resistance to all three abiotic stresses tested, with an average germination of 60% even under extremely unfavourable conditions of 30°C and 300 mM NaCl, where most modern varieties have difficulty germinating (Ibrahim et al., 2016; Zheng et al., 2009; Saboora and Kiarostami, 2006). The strong tolerance evidenced by traditional varieties may be due to several reasons. Firstly, a very ancient origin, in the case of Timilia and Einkorn, which allowed strong co-evolution and adaptation even in suboptimal and marginal areas (De Cillis, 1927). Second, their greater genetic diversity due to little or no breeding to which these varieties have been subjected over the years (Ghiselli et al., 2016). Recent studies suggest that modern cultivars have lost the Nax1 and Nax2 genes during genetic improvement (James et al., 2011), which are probably the genetic origin of the strong tolerance of Einkorn, Verna and Timilia. With the same osmotic potential in drought and salt stress tests, it was possible to isolate and verify the toxic effect of the ions on germination. First, it is immediately noticeable that the toxic effect of the ions is not constant between temperatures, but that the harmful influence of the ion linearly increases with temperature. Indeed, at 30°C, 30% and 15% of the non-germinated seeds of the modern and traditional varieties, respectively, are due to the toxic effect of the ions. The harmful interaction between ions and high temperatures is mainly due to the latter, as high temperatures cause high transpiration and greater ion uptake and production of ROS (Suzuki et al., 2016). Timilia, Einkorn and Verna also showed strong resistance to oxidative stress, which is caused at the physiological level by the greater retention capacity of Na+ ions (Adem et al., 2014).

Our results are consistent with those in the literature (Maucieri et al., 2018; Ren et al., 2005), which indicate greater germination percentages and tolerance of traditional wheat and rice varieties over modern varieties. All the three stress factors tested, especially salt stress, reduced and slowed down germination of all varieties tested, but with less intensity in Verna, Einkorn and Emmer than in Senatore Cappelli and Claudio. A slowed and delayed germination, as observed in Claudio, could lead to pronounced heterogeneity in plant height, resulting in greater mechanisation of operations such as harvesting, application of herbicides, pesticides and fertilisers (Amicabile, 2016).

In this part of the project, the great tolerance of traditional varieties to abiotic stress factors was highlighted. This agronomic trait coupled with their high genetic variability, makes them excellent varieties to be used in breeding programmes to create varieties with high forage value and agronomy. In this sense, it is interesting to highlight that traditional varieties are at the centre of a particular method of genetic improvement, Evolutionary Genetic Improvement, in which a mixture of selected seeds is sown in the field instead of pure varieties (Ceccarelli, 2016). Thanks to the great genetic diversity and spontaneous hybridisation between varieties, the seeds produced are not genetically identical to those sown. The seed obtained from this mixture is called an evolutionary population (Ceccarelli, 2016). In contrast, in conventional genetic improvement, new cereal varieties are selected by researchers in experimental stations in a process that takes decades, and only at the end of this time are the final products used in farmers' fields (Ceccarelli, 2016).

The great ability of traditional varieties to germinate even under high stress makes these varieties very important to meet all the challenges of 21st century agriculture (Casanas et al., 2017). Jarvis et al. (2016) stated that traditional varieties will be crucial for future food security, because their reintroduction enables an exponential increase in biodiversity within and between agro-ecosystems. Indeed, ecosystems with high agrobiodiversity are characterised by greater resilience and resistance to climate change, as the cultivation of traditional varieties allows for constant production over time, even when exposed to increasing stress (Kahane et al., 2013). This characteristic is particularly important for developing countries, which are increasingly plagued by climate change and which, above all, do not have high resources and inputs to cope with and mitigate such phenomena. Moreover, their use has another advantage, because the seeds of traditional varieties are independent of the current oligopoly of multinational seed companies. Indeed, these large companies determine the management of seeds by ignoring the needs of producers and consumers and focusing only on profit (Aerni, 2011), forcing small farmers without large resources to grow only a few varieties or to use inferior seeds, leading to health problems (Altieri, 2009). The monopoly of seed companies serves to standardise and homogenise the market, but above all to reduce biodiversity and territorial specificities (Campbell and Veteto, 2015).

Based on the results collected, the varieties analysed can be divided into three categories of use: Restoration and upgrading of degraded areas (Verna, Einkorn, Timilia, Turanico), moderately polluted areas (Emmer, Gentil Rosso), non-polluted areas (Senatore Cappelli, Claudio).

Further studies need to be carried out to test and detect other varieties to exploit and preserve this ancient genetic heritage. Indeed, understanding the impact of abiotic stresses on crop production and how different crop varieties respond to different stress can assure crop productivity, while avoiding degradation of arable lands and preserving natural resources.

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Growth and biomass of Traditional Varieties subjected to salt stress

Abstract

Feeding the entire, ever-growing world population will be one of the fundamental problems of the near future. However, climate change stresses specifically, and soil salinization in particular will have a massive impact on agricultural production. The objective of this study is to assess the effect of salt at the seedling phenological stages by measuring the growth and accumulation of biomass. For this research the following traditional varieties have been selected and tested, i.e., “Einkorn” *Triticum monococcum* L, “Emmer” *T. dicoccum* L., *T. durum* Desf. var. Senatore Cappelli, *T. aestivum* L. var. Verna, *T. durum* Desf. var. Timilia, *T. aestivum* L. var. Gentil Rosso, *T. turanicum* Jakubz., and one modern variety, the *T. durum* Desf. var. Claudio. This part of the project is consistent with the precedent and confirms the chapter in vitro tests. Indeed, it has been shown that the traditional varieties show, on average, greater growth and biomass accumulation than the modern variety Claudio.

Introduction

Food security is expected to become an increasingly important issue in the coming decades. While agricultural productivity has reached a plateau in many countries (Cassman et al., 2010), the world population continues to grow (Godfray et al., 2010; Bommarco et al., 2013) and producing enough food to feed the world's population is becoming increasingly challenging (UN, 2015). In addition, food production is threatened by climate change and future scarcity of non-renewable resources (Lobell and Gourdji, 2012; Vermeulen et al., 2012; EU, 2015; Myers et al., 2017).

Climate change, including sudden changes in temperature and precipitation and soil salinization, is expected to globally affect conventional agricultural production (Zalidis et al., 2002; Scotti et al., 2015), with an estimated 33% decline in yields and quality (Gammans et al., 2017). Increase in soil salinity already affects more than 40% of Mediterranean soils (Nedjimi, 2014), due to seawater intrusion into freshwater aquifers, scarce winter rainfall, irrigation with brackish water and the use of pesticides and fertilisers (Rana and Katerji, 2000). In addition, in oversalted soils there is reinforcing feedback characterized by a compact surface layer that minimizes fertilizer uptake and increases salinization and nutrient runoff (Durán Zuazo and Rodríguez Pleguezelo, 2009). Salt accumulation affects 10 million hectares of land each year, resulting in 50 million tons less crop (Machado and Serralheiro, 2017).

Soil salinization is a very extensive problem, specifically 932.2 Mha globally affected (Rengasamy, 2006), and it is recognized as one of the major soil degradation drivers, affecting 34.19 10% of the total irrigated land (Aquastat, 2016). Global soil salinization hotspots include Pakistan, China, United States, India, Argentina, Sudan and many countries in Central and Western Asia (Aquastat, 2016; Ghassemi et al., 1995). Soil salinization is increasingly become a disruptive issue in Europe as well, especially in coastal areas (Daliakopoulos et al., 2016).

Salinity affects crop growth in several ways, such as osmotic effects, specific ion toxicity and/or nutritional disturbances (Gorham et al., 1985; Munns and Tester, 2008). For example, the salt accumulation in roots reduces the ability of extracting water from the soil, leading to imbalances in plant nutrition (Hasegawa et al., 2000; Munns, 2002; Munns and Tester, 2008; Wang et al., 2020). High soil salinity has been found to trigger the formation of reactive oxygen species (ROS), which can compromise the integrity of biological membranes (Foyer, 2018). ROS led to a slowdown in the absorption of carbon dioxide, resulting in low CO_2 concentration at the chloroplast level, which inhibits carbon fixation, and high energetic excitation of chloroplasts with a consequent reduction in biomass accumulation (Foyer, 2018). In soils with moderate to high salt concentrations, biomass reductions of up to 50% have been reported; in severe cases, agricultural production is no longer practicable (Foyer, 2018). Recent estimates suggest that soil salinization will reduce yields of forage pastures in affected regions by 20-36%, resulting in huge economic losses estimated at €36 billion to the agricultural sector (Giridhar and Samireddypalle, 2015).

Due to increasing pressure on freshwater resources, the identification of salt-adapted crop varieties, i.e., varieties that are able to survive under saline conditions and complete their growth cycle with acceptable growth and yield, is a new challenge for use in salt-affected areas (Corwin, 2021).

One potential strategy for identifying crops that can survive under limiting conditions is to explore the salt tolerance of traditional cereal varieties, commonly referred to as landraces, which have adapted to a wide range of adverse conditions during their domestication and subsequent cultivation (Schmidt et al., 2019). Traditional varieties have been shown to have low nutrient requirements and high resistance to environmental stress (Casañas et al., 2017). They are also adaptable to local, often marginal environments where modern varieties lose their competitive advantage (Villa et al., 2005). Despite these characteristics, the use of traditional varieties is declining, mainly due to their lower productivity compared to modern high-yielding varieties (Moudry et al., 2012; Casañas et al., 2017). Since the traditional varieties showed remarkable tolerance to salt stress at the germination stage, this study assessed the effect of salt at the seedling phenological stages by measuring the growth and accumulation of biomass.

Methods

Seed collection

To test and compare salt stress tolerance, we selected seeds of seven ancient varieties i.e., "Einkorn" *Triticum monococcum* L, "Emmer" *T. dicoccum* L., *T. durum* Desf. var. Senatore Cappelli, *T. aestivum* L. var. Verna, *T. durum* Desf. var. Timilia, *T. aestivum* L. var. Gentil Rosso, *T. turanicum*

Jakubz., 1947 and one modern variety, *T. durum* Desf. var. Claudio. Seeds were obtained from the Rural Seed Network of Umbria and Los Prados Company. The chosen eight cereal varieties are briefly descripted in Table 1. The tested varieties were stored at 18 ° C and 40% humidity in the storage cell of Ca'Foscari University.

Table 1 Description of the tested varieties (De Cillis, 1927; Ghiselli et al., 2016; Trebbi et al., 2011)

Variety	Species	Registration Year	Country	Pedigree
Verna	*Triticum aestivum* L.	1953	Italy	Est Mottin/Mont Calme 245
Einkorn	*Triticum monococcum L.*	/	Italy	/
Emmer	*Triticum dicoccum L.*	/	Italy	/
Senatore Cappelli	*Triticum durum Desf.*	1930	Italy	Selection from Jennah Rhetifa
Claudio	*Triticum durum Desf.*	1999	Italy	CIMMYT35/Durango//IS1938/Grazia
Timilia	*Triticum durum Desf.*	/	Italy	/
Turanico	*Triticum turanicum Jakubz., 1947*	1921	Italy	/
Gentil Rosso	*Triticum aestivum L.*	1850	Italy	/

Seedling growth tests

The growth studies were conducted in the greenhouse of the Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice.

To comprehensively analyse how salt affects the growth and the biomass accumulated, seedlings of the traditional varieties and the var. Claudio have been germinated at 100, 200 and 300 mM of NaCl and then transplanted into pots containing common topsoil. Seedlings of the varieties have been watered with 0 (fresh water), 100, 200 and 300 mM of NaCl (40 replicates for each salt regime and each variety; n=320 seedlings). After 8 weeks we measured the stem diameter at the first node (mm) and plant height (cm) on 10 randomly selected for each variety and condition, following Cornelissen et al. (2003). Moreover, as a proxy for the biomass accumulated, for each condition and variety (n=100), we collected 4 young fully expanded and undamaged leaves from 10 replicates randomly chosen. Leaves were then placed in an oven for 48 hours at 80 ° C and weighed to obtain the leaf dry weight (LDW) (g). LDW values were then averaged by condition and variety.

Data analysis

For each cultivar and salt concentration, stem diameter, plant height and LDW values were compared using the one-way PERMANOVA test with 999 randomisations, using salt concentration as the grouping variable. Fisher's Least Significant Difference (LSD) was then performed to identify significant differences in the calculated parameters between NaCl concentrations.

Results

All the tested varieties were significantly (LSD test, p-value < 0.05) affected by the presence of salt in the growth medium (Tables 2, 3 and 4). The traditional cultivars with fresh water had an average height of 45.81 ± cm, an average diameter of 0.30 ± cm and a LDW of 0.061 ± g, while Claudio without salt had an average height of 40.8, an average diameter of 0.301 and a leaf biomass of 0.034 g. The presence of salt significantly reduced all measured parameters in the modern Claudio cultivars. At only 100 mM NaCl, the average height of the Claudio seedlings decreased by 43%, and at 300 mM NaCl by as much as 52%. In addition, when leaf biomass was measured, a greater percentage loss due to salt stress was observed, from 50% at 100 mM NaCl to 70% at 300 mM NaCl. As observed in the germination phase, the traditional cultivars showed a greater resistance to salt stress, namely a lower percentage loss compared to Claudio. In fact, they showed a reduction in height and diameter from 30% at 100 mM NaCl to a maximum reduction of 45% at 300 mM NaCl. As in the modern cultivar Claudio, biomass was more affected by salt stress in the traditional cultivars. An average reduction of 60% was observed at 300 mM. However, the greater resistance among the traditional varieties was heterogeneous, as the Senatore Cappelli variety showed greater susceptibility to salt stress, as in the germination phase, and percentage losses in the parameters tested (58%) were observed at 300 mM, which were comparable to those of the Claudio variety. In contrast, Einkorn, Turanico, Verna and Timilia resulted exceptionally resistant to salt stress. At 300 mM NaCl, they showed a decrease in height and diameter of 35 %, 45% and 50 % of biomass, respectively. The traditional varieties Emmer and Gentil rosso, on the other hand, showed an intermediate behavior compared to the varieties described above. In fact, at 300 mM NaCl, they showed a 48% reduction in height and diameter and a 60% reduction in LDW.

Table 2 Means ± SD of Diameter of the tested varieties. Values followed by different capital letters significantly differ across varieties at the same NaCl concentration (Fisher LSD test). Values followed by small letters significantly differ across salt concentrations within each variety (Fisher LSD test).

	Salt Concentration (mM)			
	0 mM NaCl	100 mM NaCl	200 mM NaCl	300 mM NaCl
Claudio	0.301±0.015Aa	0.143±0.007Ab	0.134±0.007Ac	0.125±0.006Ad
Einkorn	0.318±0.016B a	0.204±0.01B b	0.196±0.01B c	0.178±0.009B d
Timilia	0.306±0.015Ca	0.197±0.01Cb	0.189±0.009Cc	0.172±0.009Cd
Verna	0.3±0.015Da	0.193±0.01Db	0.185±0.009Dc	0.168±0.008Dd
Senatore Cappelli	0.326±0.016Ea	0.19±0.01Eb	0.182±0.009Ec	0.141±0.007Ed
Emmer	0.297±0.015Fa	0.189±0.009Fb	0.177±0.009Fc	0.154±0.008Fd
Gentil Rosso	0.291±0.015Ga	0.185±0.009Gb	0.174±0.009Gc	0.151±0.008Gd
Turanico	0.294±0.015Ha	0.189±0.009Hb	0.182±0.009Hc	0.165±0.008Hd

Table 3 Means ± SD of Height of the tested varieties. Values followed by different capital letters significantly differ across varieties at the same NaCl concentration (Fisher LSD test). Values followed by small letters significantly differ across salt concentrations within each variety (Fisher LSD test).

	Salt Concentration (mM)			
	0 mM NaCl	100 mM NaCl	200 mM NaCl	300 mM NaCl
Claudio	40.8±2.04Aa	23.22±1.161Ab	21.18±1.059Ac	17.09±0.855Ad
Einkorn	46.1±2.305Ba	35.34±1.767Bb	31.87±1.594B c	31.84±1.592B d
Timilia	46.45±2.323Ca	31.76±1.588Cb	30.91±1.546Cc	29.18±1.459Cd
Verna	46.33±2.317Da	35.19±1.76Db	31.18±1.559Dc	30.13±1.507Dd
Senatore Cappelli	45.63±2.282Ea	34.76±1.738Eb	30.64±1.532Ec	19.15±0.958Ed
Emmer	45.86±2.293Fa	34.44±1.722Fb	29.84±1.492Fc	20.77±1.039Fd
Gentil Rosso	44.94±2.247Ga	33.75±1.688Gb	29.25±1.463Gc	20.35±1.018Gd
Turanico	45.41±2.271Ha	34.48±1.724Hb	30.55±1.528Hc	29.53±1.477Hd

Table 9 Means ± SD of LWD of the tested varieties. Values followed by different capital letters significantly differ across varieties at the same NaCl concentration (Fisher LSD test). Values followed by small letters significantly differ across salt concentrations within each variety (Fisher LSD test).

	Salt Concentration (mM)			
	0 mM NaCl	100 mM NaCl	200 mM NaCl	300 mM NaCl
Claudio	0.035±0.002Aa	0.014±0.001Ab	0.013±0.001Ac	0.01±0.001Ad
Einkorn	0.064±0.003B a	0.033±0.002B b	0.032±0.002B c	0.023±0.001B d
Timilia	0.064±0.003Ca	0.03±0.002Cb	0.029±0.001Cc	0.022±0.001Cd
Verna	0.064±0.003Da	0.033±0.002Db	0.031±0.002Dc	0.021±0.001Dd
Senatore Cappelli	0.063±0.003Ea	0.032±0.002Eb	0.031±0.002Ec	0.014±0.001Ed
Emmer	0.063±0.003Fa	0.032±0.002Fb	0.03±0.002Fc	0.015±0.001Fd
Gentil Rosso	0.062±0.003Ga	0.031±0.002Gb	0.029±0.001Gc	0.014±0.001Gd
Turanico	0.063±0.003Ha	0.032±0.002Hb	0.031±0.002Hc	0.032±0.002Hd

Discussion

Several studies have shown that traditional varieties are more dynamic and capable of development than genetically homogeneous varieties due to their high genetic diversity. These are crucial traits that could improve the resilience and resistance of agroecosystems (Jarvis et al, 2016; Veteläinen et al 2009; Nevo and Chen, 2010). This part of the project is consistent with and confirms the in vitro tests carried out on germination, the preceding phenological stage. Indeed, it has been shown that the traditional varieties show, on average, greater growth and biomass accumulation than the modern variety Claudio. However, Senatore Cappelli showed susceptibility and a percentage loss of height and leaf biomass comparable to that of the modern variety Claudio. Conversely, the strong tolerance of Timilia, Einkorn, Turanico and Verna to salt stress was also confirmed in the subsequent phenological phases. In fact, a relatively small reduction in height and biomass of about 35% was observed in these cultivars at 300 mM. This trait can be explained by a higher water uptake capacity and a greater ability to adjust the osmotic potential and control the ROS cell level, allowing stable growth even under the highest salt stress (Miransari and Smith, 2019). Senatore Cappelli and Claudio, the less salt-tolerant varieties, have a common origin, since Claudio, like most modern durum wheat varieties, originated from the mutation of Senatore Cappelli by irradiation with gamma rays and neutrons (Amicabile , 2016). In contrast, Verna, Emmer and Einkorn (De Cillis, 1927) and Timilia (Maucieri 2018) were not bred and underwent less intensive genetic improvement. The possible loss of gene loci responsible for adaptation to salinity, such as the Nax1 and Nax2 loci (James et al, 2011), during genetic improvement could therefore be the possible cause of Claudio and Senatore Cappelli's salt sensitivity.

Our results are consistent with the existing literature and show how salt stress decisively affects biomass accumulation in modern cultivars. Indeed, several modern cultivars (Misr-2, Sakh-95, Pak, Lu, Mongibello, p.24, SH -97) show a strong reduction in biomass accumulation even at low salt concentrations (Maucieri et al., 2018; Hameed et al., 2008; Yassin et al., 2019; Khan et al., 2006). This part of the project confirmed how saline stress has the capability of affecting growth and accumulation of biomass in the phenological stages following emergence (Almanosuri et al., 2001). Furthermore, our study shows how salt stress predominantly influences the accumulation of green biomass compared to the other measured parameters. This phenomenon is due to the strong influence of salt on the overall photosynthetic process and biomass accumulation, as the affinity of Rubisco for CO_2 decreases, reducing carbon fixation (Foyer, 2018). The critical reduction can be a critical issue for conventional forage producers who need high biomass crops to support the livestock sector (Gammans et al., 2017). The great capacity of traditional varieties to grow in high soil salinity makes them very important in meeting the challenges that the agricultural sector will have to face in the future, and thus represents a sustainable way of mitigating soil salinisation and minimising economic losses (Lafitte et al., 2004; Anzooman et al., 2018); this may ultimately help to alleviate concerns about future food security that have arisen due to the increased sensitivity of modern varieties (Khourya et al., 2014). Furthermore, as their cultivation requires less fertiliser and pesticides, which are the main causes of secondary salinisation (Corwin, 2021), they can help mitigate soil salinisation. Finally, the use of traditional genotypes can also be an important resource for breeders (Maucieri et al., 2018; Del Vecchio et al. 2019), as they represent an exceptional source of resistance and diversity, both at the genetic and landscape level.

Further studies are needed to test and discover other varieties to exploit and conserve this ancient gene pool. By understanding how soil salinisation affects crop production and how different crop varieties respond to salt stress, we can ensure crop productivity while avoiding crop degradation and conserving natural resources.

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Assessment of the mitigating capacity of saline stress by selected yeast strains

Abstract

An innovative sustainable strategy to mitigate climate change stresses, and especially soil salinization, is the exploitation of Microbial biostimulants. Microbial biostimulants (Bacteria, Yeast and Fungi) are substances capable of improving crop productivity and quality, increasing the availability of nutrients in the soil, ameliorating plant use efficiency of nutrients, and promoting the degradation and humification of organic substances in soils. The aim of this study, indeed, is to evaluate the salt and heat stress mitigating capability of 6 yeast strains, i.e., *Aureobasidium pullulan, Kluyveromyces marxianus, Debaryomyces hansenii, Zygosaccharomyces rouxii, Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae*, and their possible utilization in order to mitigate and improve cultivation under salt stress. The study focused on 3 ancient varieties (*Triticum durum* Desf. var. Timilia, *T. durum* Desf. var. Senatore Cappelli and "Einkorn" *T. monococcum* L) and one modern crop (*T. durum* Desf var. Claudio). To test salt stress mitigating capacity, germination tests were carried out 300 mM. The varieties showed significantly different salt tolerances. The 3 halophilous yeast strains show a marked mitigating capability, with an average increase in germination of 20 %. *Zygosaccharomyces rouxii* showed an outstanding mitigant effect, about 36 %, coupled with Senatore Cappelli seeds. In order to evaluate thermal stress mitigating capacity germination tests were carried out 30°C. However, the 3 thermotolerant yeast strains led to no significant stress relief effect. The high mitigating capability observed for *Debaryomyces hansenii, Zygosaccharomyces rouxii and Rhodotorula mucilaginosa* suggests that the exploitation of microbial biostimulants could be a pivotal resource to face future agricultural challenges and ensure future food security.

Introduction

Since the end of the eighteenth century and the Malthus essay, Food Security is one and will be the major issues of global society (Malthus et al., 1798). Currently, more than a billion people do not have access to a safe and long-lasting source of food (FAO, 2014). To overcome and mitigate this phenomenon the “Green Revolution” took place (Pingali, 2012) i.e. the complete industrialization and mechanization of agricultural sector in order to increase crop yield and reduce world hunger (Tilman et al., 2002).

However, this farming model is unsustainable and one of the main drivers of Climate Change indeed, it is the source of 25% of all greenhouse gas emissions and consumes large amounts of natural resources: 70% of the world's freshwater (FAO, 2017) and almost 40% of arable land (Altieri, 2009). However, weather and climate conditions have a significant impact on agricultural systems, as they are vulnerable to adverse and extreme conditions. Moreover, 200 million more people could be at risk of hunger by 2100 (Schmidhuber and Tubiello, 2007). (Schmidhuber and Tubiello, 2007). The physiological and macromorphological detrimental effects of abiotic stress due to climate change have been described in detail in the previous chapters. It is therefore necessary to find new, profitable production models such as Agroecology model (Moore, 2015). Agroecology, conceives and applies the dynamics of ecological ecosystems to the agricultural system ecological, in the near future it will play a fundamental role in adapting agriculture to the ongoing climate change (Altieri 2009). The main principle of agroecology is the enhancement of agrobiodiversity within and between different agro-ecosystems to increase crop yields, but more importantly, their resilience and resistance (Gliessman et al., 1998). Indeed, agrobiodiversity can enhance the adaptive capacity of agroecosystems to biotic and abiotic stressors, thus ensuring food security in a climate change scenario (Lennè and Wood, 2011).

An agroecological innovative sustainable strategy to mitigate climate change stresses, and especially soil salinization, is the exploitation of Microbial biostimulants (Fedotov et al., 2017). Microbial biostimulants (Bacteria, Yeast and Fungi) are substances capable of improving crop productivity and quality, increasing the availability of nutrients in the soil, ameliorating plant use efficiency of nutrients, and promoting the degradation and humification of organic substances in soils (Caradonia et al., 2019; Parađiković et al., 2011). Soil yeasts were found to accelerate the development of seeds of common crops (wheat, barley, and rye) (Fedotov et al., 2017). Biostimulants such as yeast and bacteria, have been also suggested to induce the activation of an induces systemic tolerance, thereby allowing plants to tolerate or attenuate detrimental effects of abiotic stresses (Yang et al., 2009).

Another sustainable strategy in mitigating climate change on the agricultural sector is the exploitation of stress tolerant crop varieties (Maucieri et al., 2018), i.e., varieties able to survive under stress conditions and complete their growth cycle with an acceptable growth and yield. Indeed, traditional crop varieties have been proved to have a great resistance to environmental stress, superior nutritional content, and great adaptability due to their higher genetic diversity (Casañas et al., 2017). Once widespread, the use of traditional cereal has been decreasing, due to their lower productivity compared to modern varieties (Moudry et al., 2012; Casañas et al., 2017).

Given that, the purpose of this work was to evaluate the capability of tolerant yeast autolyzates in mitigating saline and thermal stress in traditional crop varieties. Since seed germination is a critical phase in the lifecycle of higher plants, we evaluated the effects of yeast autolyzates on seed germination ability, which is regarded as a rapid and efficient method to assess stress tolerance (Ashraf et al., 1987).

Methods

Seed Collection

Salt stress mitigation has been studied through the selection of three traditional varieties seeds, i.e., *Triticum durum* Desf. var. Senatore Cappelli, "Einkorn" *T. monococcum* L., *T. durum* Desf. var. Timilia and one modern variety, *T. durum* Desf. var. Claudio. Seeds were obtained from the Rural Seed Network of Umbria and Los Prados Company in Italy. We chose to test these varieties because, as observed and verified in the previous chapter of the thesis, they represent the whole germination spectrum, from the more sensitive ones (Claudio and Senatore Cappelli) to the more resistant ones (Timilia and Einkorn). All the selected varieties were harvested in July 2018 and then placed in the storage cell (18 °C, 40% humidity). The chosen three cereal varieties were briefly descripted in Table 1.

Table 10 Description of the tested varieties (De Cillis, 1927; Ghiselli et al., 2016; Trebbi et al., 2011)

Variety	Species	Registration Year	Country	Pedigree
Einkorn	*Triticum monococcum L.*	/	Italy	/
Senatore Cappelli	*Triticum durum Desf.*	1930	Italy	Selection from Jennah Rhetifa
Claudio	*Triticum durum Desf.*	1999	Italy	CIMMYT35/Durango//IS1938/Grazia
Timilia	*Triticum durum Desf.*	/	Italy	/

Yeast Selection and Autolyzates preparation

To identify stress-resistant yeast strains, we conducted an extensive literature search. The literature search was conducted in the ISI Web of Knowledge database using "yeast", "halophilic", "thermophilic", "tolerance", "salt stress" and "salt stress" as keywords.

The results obtained were cross-checked with the gene bank of the Department of Agricultural Microbiology of the University of Perugia to select the 6 yeast strains described in Table 2.

Yeast colonies were grown for 14 days in YPD Yeast Extract–Peptone–Dextrose culture medium and centrifuged at 13000 rpm. Autolyzates were obtained through the supernatant filtration (Fedotov et al., 2017).

Table 11 Description of the tested Yeast strain

Yeast Strain	**Registration Year**	**Adaptation**
Aureobasidium pullulan (de Bary) G. Arnaud	1918	Thermophilic
Kluyveromyces marxianus Van der Walt	1965	Thermophilic
Saccharomyces cerevisiae Meyen ex E.C. Hansen	1883	Thermophilic
Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij	1984	Halophilous
Zygosaccharomyces rouxii (Boutroux) Yarrow	1977	Halophilous
Rhodotorula mucilaginosa (Fresen.) F.C.Harrison	1928	Halophilous

Germination Tests

The analysis of viability and the sterilisation of the seeds were carried out as described in the previous chapter. For each crop variety and condition, 10 replicates of 20 seeds each were sown in a sterile environment on two sheets of Whatman No.1 filter paper in 9-cm diameter Petri dishes.

To analyse salt stress mitigation, seeds were exposed to 300 mM of NaCl and 2 ml of halophilous yeast autolyzates has been applied. Seeds were incubated into a growth chamber (Vetrotecnica UW134 AT1.69 CAJ4492Y Opn pXS HG TCRH2SS EV Spec) at 20 ± 1,5°C, which represents the thermal optimum for the germination of cereal varieties (Orsenigo et al., 2017; Bicksler, 2011), for 30 days. Germination was scored every 24 h for the entire duration of the tests. To evaluate the thermal stress mitigation, seeds of traditional varieties were germinated at 30 ° C and then 2 ml of thermotolerant yeast autolysate were administered. Seed scoring methodology is the same previously described.

Data analysis

To analyse the mitigation of yeast autolysates on the germination of the four crop varieties under abiotic stress, we calculated three parameters (Joosen et al., 2010), averaged by the 10 replicates for each tested condition: a) germination percentage, calculated as the number of germinated seeds on the total number of sown seeds, multiplied by one hundred; b) median germination time (T50), namely the time (in days) required to achieve 50% of germination; c) germination delay, as the number of days until the first germination occurred.

$$T50 = t1 + \frac{\left[\left(\frac{N}{2} - n1\right)(t1 - t2)\right]}{n1 - n2}$$

For each variety and yeasts autolysates, germination percentage, T50 and germination delay were compared by one-way PERMANOVA test with 999 randomizations, using yeast strain as grouping variables. Fisher's least significant difference (LSD) was then performed to identify significant differences of the calculated parameters among varieties.

Results

Salt concentration of 300 mM of NaCl significantly (PERMANOVA test; $p < 0.05$; Table 1) affected all the germination parameters (germination percentage, T50 and germination delay) among the varieties. However, the tested crops were differently affected by salinity stress; Claudio and Senatore Cappelli proved to be the most sensitive with an average germination percentage between 2 out of 42.25 ± 0.88 at 300 mM. Conversely, Timilia and Einkorn showed a marked salt tolerance (77.25 ± 1.50, averaged between the two varieties).

The administration of 2 ml of halophilous yeast autolysates led to significant results (PERMANOVA test; $p < 0.05$, Table 1-3), with an average increase of 19% in the germination percentage. Both germination delay and median germination time (T50) respectively decreased by 25% and 36%.

Among the tested varieties, Senatore Cappelli showed to be the most sensitive to the salt stress mitigating effect exerted by the yeast strain, and all the three parameters (Germination percentage, Germination Delay, Medium Germination Time) increased by an average of 36.5 %. Claudio variety, also, was highly influenced by the yeast autolysates, showing a germination percentage increase of 25 %.

Among the halophilic yeasts, *Zygosaccharomyces rouxii* significantly (PERMANOVA test; $p < 0.05$, Table 3) showed a greater ability to reduce salt stress in all test cultivars, resulting in an average 23 % increase in seed germination. In contrast, *R. mucilaginosa* and *D. hansenii* increase germination by 18 % and 16 %, respectively.

The germination of all the varieties has been significantly affected (LSD test; $p < 0.05$, Table 5) by the temperature of 30 °C. As described above, the germination test showed an heterogeneous response to the heat stress. Based on the results of the previous chapter, Timilia, Einkorn and Senatore Cappelli are characterised by greater heat tolerance compared to the modern Claudio varieties. However, the application of the autolysates of three thermotolerant yeast strain led to no significant mitigating effect (PERMANOVA test; $p < 0.05$, Table 4).

Table 3 PERMANOVA results of the germination percentage, median germination time (T50) and germination delay under 300 mM of NaCl with different yeast for the tested varieties.

		Germination Percentage			Median Germination Time (T50)			Germination Delay		
		DF	F Model	P-value	DF	F Model	P-value	DF	F Model	P-value
Claudio	Yeast	3	90.574	0.001	3	301.226	0.001	3	806.67	0.001
Timilia	Yeast	3	56.817	0.001	3	2150.37	0.001	3	107.836	0.001
Einkorn	Yeast	3	19.954	0.001	3	13451.5	0.001	3	415.95	0.001
Senatore Cappelli	Yeast	3	114.742	0.001	3	2105.77	0.001	3	2397.58	0.001
	Residuals	36			36			36		

Table 4 PERMANOVA results of the germination percentage, median germination time (T50) and germination delay under 300 mM of NaCl among the tested varieties.

	Germination Percentage			Median Germination Time			Germination Delay		
	DF	F Model	P-value	DF	F Model	P-value	DF	F Model	P-value
Variety	3	37.219	0.001	3	421.67	0.001	3	1076.1	0.001
Yeast Strain	3	135.180	0.001	3	1860.13	0.001	3	8897.4	0.001
Variety x Yeast	9	20.089	0.001	9	101.57	0.001	9	393.0	0.001
Residuals	144			144			144		

Table 5 Means ± SD for germination percentages, median germination time (T50) and germination delay of the tested varieties Values followed by different capital letters significantly differ yeast Strain (Fisher LSD test). Values followed by small letters significantly differ across yeast strain within each variety (Fisher LSD test).

		Yeast Strain				
	Variety	0	300 mM NaCl	*Z. rouxii*	*D. hansenii*	*R.mucilaginosa*
Germination Percentage (%)	Claudio (Modern Variety)	90.50 ± 6.34Aa	39.00 ± 5.94Ab	64.00 ± 3.85 Ac	60.25 ± 7.14Ad	52.50 ± 7.83Ae
	Timilia	97.85 ± 2.41Ba	76.25 ± 5.77 Bb	97.50 ± 3.33 Ba	88.75 ± 5.86Bc	91.25 ± 6.25Bd
	Einkorn	99.00 ± 2.58Ca	78.25 ± 8.64Cb	90.50 ± 2.5 Cc	90.50 ± 2.41Ca	92.50 ± 5.50Bb
	Senatore Cappelli	97.00 ± 4.83Ba	45.5 ± 8.83Dd	82.00 ± 5.55 Da	65.60 ± 4.67Db	78.50 ± 6.23 Cb
Median Germination Time	Claudio (Modern Variety)	1.00 ± 0 Aa	2.71 ± 0.44 Ab	1.10 ± 0.01 Ac	1.30 ± 0.20 Ad	1.20 ± 0.40 Ae
	Timilia	0.50 ± 0.09 Ba	1.50 ± 0.43 Bb	0.70 ± 0.1 Bc	0.73 ± 0.45 Bc	0.71 ± 0.37 Bc
	Einkorn	0.50 ± 0.03 Ba	0.50 ± 0.02Ca	0.50 ± 0.02 Ca	0.50 ± 0.01 Ca	0.51 ± 0.07 Ca
	Senatore Cappelli	0.60 ± 0.04 Ba	2.49 ± 0.79 Bb	0.60 ± 0.1 Da	0.63 ± 0.31 Da	0.62 ± 0.44 Da
Germination Delay (Days)	Claudio (Modern Variety)	0.50 ±0Aa	5.00 ± 0 Ab	1 ± 0.16 Ac	2.10 ± 0.31Ad	1.70 ± 0.94 Ae
	Timilia	0.50 ± 0Aa	2.00 ± 0 Bb	0.50 ± 0Ba	0.50 ± 0Ba	0.50 ± 0.42 Ba
	Einkorn	0.50 ± 0Aa	1.00 ± 0 Cb	0.50 ± 0Ba	0.50 ± 0Ba	1.00 ± 0 Ca
	Senatore Cappelli	0.50 ± 0Aa	2.00 ± 0.51 Bb	0.50 ± 0Ba	0.50 ± 0.51Ba	0.70± 0Bc

Table 6 PERMANOVA results of the germination percentage, median germination time (T50) and germination delay under 30°C among the yeasts and tested varieties.

	Germination Percentage			Median Germination Time			Germination Delay		
	DF	F Model	P-value	DF	F Model	P-value	DF	F Model	P-value
Variety	3	15.831	0.001	3	421.67	0.001	3	1076.1	0.001
Yeast Strain	3	1.134	0.339	3	1.860	0.452	3	1.197	0.364
Variety x Yeast	9	0.7833	0.628	9	0.578	0.626	9	0.393	0.477
Residuals	144			144			144		

Table 7 Means ± SD for germination percentages, median germination time (T50) and germination delay of the tested varieties Values followed by different capital letters significantly differ yeast Strain (Fisher LSD test). Values followed by small letters significantly differ across yeast strain within each variety (Fisher LSD test).

		Yeast Strain				
	Variety	0	30 °C	*S. cerevisiae*	*K. marxianus*	*A. pullulans*
Germination Percentage (%)	Claudio (Modern Variety)	90.50 ± 6.34Aa	82.25 ± 7.94Ab	85.00 ± 3.85 Ac	87.50 ± 7.14Ad	85 ± 7.83Ae
	Timilia	97.85 ± 1.41Ba	96 ± 1.77 Bb	98.75 ± 1.33 Ba	99 ± 0.86Bc	96.50 ± 6.25Bd
	Einkorn	99.00 ± 1.58Ca	97 ± 2.64Cb	98.75 ± 0.5 Cc	97.50 ± 2.41Ca	99 ± 0.50Bb
	Senatore Cappelli	97.00 ± 1.83Ba	95.5 ± 1.83Dd	96.25 ± 2.55 Da	93.75 ± 4.67Db	96.75 ± 2.23 Cb
Median Germination Time	Claudio (Modern Variety)	1.00 ± 0 Aa	2.11 ± 0.44 Ab	1.10 ± 0.01 Ac	1.30 ± 0.20 Ad	1.20 ± 0.40 Ae
	Timilia	0.50 ± 0.09 Ba	2.69 ± 0.43 Bb	0.70 ± 0.1 Bc	0.73 ± 0.45 Bc	0.71 ± 0.37 Bc
	Einkorn	0.50 ± 0.03 Ba	0.50 ± 0.02Ca	0.50 ± 0.02 Ca	0.50 ± 0.01 Ca	0.51 ± 0.07 Ca
	Senatore Cappelli	0.60 ± 0.04 Ba	2.69 ± 0.79 Bb	0.60 ± 0.1 Da	0.63 ± 0.31 Da	0.62 ± 0.44 Da
Germination Delay (Days)	Claudio (Modern Variety)	0.50 ±0Aa	1.20 ± 0.13 Ab	1 ± 0.16 Ab	1.10 ± 0.31Ab	1.12 ± 0.94 Ab
	Timilia	0.50 ± 0Aa	0.50 ±0.2 Bb	0.50 ± 0.2Bb	0.50 ± 0Bb	0.50 ± 0.42 Bb
	Einkorn	0.50 ± 0Aa	0.50 ± 0.3 Bb	0.50 ± 0.3Bb	0.50 ± 0Bb	0.50 ± 0.34 Bb
	Senatore Cappelli	0.50 ± 0Aa	0.60 ± 0.11 Cb	0.61 ± 0.4Cb	0.62 ± 0.51Cb	0.70± 0.21Cb

Discussion

Microbial biostimulants are diverse substances which improve germination and growth of plants through nutrient uptake, nutrient efficiency, and tolerance to abiotic stress (Del Buono et al., 2021). In our study, treatments with *D. hansenii*, *Z. rouxii* and *R. mucilaginosa* autolysates significantly enhanced seed germination of all the varieties under salt stress. The mitigating efficacy of yeast autolysates averaged 20 %; however, Senatore Cappelli coupled with *Z. rouxii* autolysates showed an increment 1.5 times greater than the average. Among the tested varieties Senatore Cappelli and Claudio showed the highest germination increase compared to *T. monococcum* and Timilia. These results could depend on the extreme sensitivity to saline stress of Claudio and Senatore Cappelli, dissimilar to Timilia and Einkorn which are characterized by an outstanding salt tolerance (Maucieri et al., 2018). Among the yeast strain *Z. rouxii* autolysates proved the greatest mitigating efficacy, followed by *R. mucilaginosa* and *D. hansenii*.

On the contrary, the application of thermotolerant yeast autolysates led to no significant stress relief. Further microbiological studies are needed to understand the reasons for this difference in mitigation effectiveness among the yeast strains.

Consistently with literature (Almansouri et al., 2001), in all the tested varieties, salt stress delayed and slowed down the germination, however with a heterogeneous response pattern.

However, the halophilous yeast autolysates interaction, especially *Z. rouxii*, led to an average 20% earlier germination response, this positive synergistic effect is very important because, according to Smith and Cobb (1991), faster emergence leads to higher photosynthetic activity and greater final biomass. In fact, germination 1 day earlier resulted in a significant 26% increase in final biomass.

Halophilic yeast autolysates are able to promote the build-up of biomass because they: a) reduce the salt toxic effect and especially reduce the accumulation of Na+ and Cl- in the roots (Wahome, 2001) b) promote water storage in plant tissues (Del Buono, 2021).

c) increased activity of abscisic acid synthesis (ABA). ABA is a signal involved in the response to various environmental stress factors, especially salinity Chitarra et al. (2016).

Barassi et al. (1997) found that inoculation of *Azospirillum* sp. at the germination stage can restrict Na+ influx into seeds and, in particular, improve soil water uptake. Various microbial biostimulants have already been proven to have mitigation effects on several crops. Algal extracts have been used to mitigate the toxic effects of salts in Kentucky bluegrass (*Poa pratensis L.*) (Le Mire et al., 2016). *Rhizophagus irregularis*, a strain of a symbiotic arbuscular mycorrhizal fungi, enhanced the growth potential of *Stevia rebaudiana* (Gaiero et al., 2013).

The selection of stress tolerant yeast and bacteria is a mandatory step for reducing the use of energy intensive chemical fertilizers. Indeed, an extensive utilization of microbial biostimulantss could significantly increase the crop uptake of NPK and drastically reduce fertilizer air dispersion and GHG emissions (Poss et al., 1999). According to Fiorentino et al. (2018), strains of *Trichoderma* in lettuce promote native N uptake, nutrient availability and mobilisation in both vegetables and soil.

The strains reported in this study are suitable for all agroecosystems affected by salinisation and desertification due to their marked salt tolerance (Verma et al., 2016). Moreover, their applications as microbial biostimulants for agricultural purposes could be diverse. Indeed, the use of yeast as a seed dressing could be a crucial resource for agroecosystems. Sgolastra et al. (2017) found that conventional seed dressing, which consists of a mixture of pesticides and fungicides, leads to massive pollinator and bee declines, abnormal behaviour and weakening of bee colonies. Indeed, the use of microbial biostimulants could contribute to the maintenance of agro-ecosystem ecological balance, minimizing the use of pesticides and/or heavy metals in agriculture and increasing biodiversity and soil properties (Del Buono, 2021; Van Oosten et al., 2017).

According to Tejada et al., (2011) the utilization of microbial biostimulants lead to an increased quality of soil organic matter content and above all promote vegetation biodiversity in order to reduce soil erosion.

Further studies need to be carried out to test and discover other yeast and bacterial species to exploit their outstanding properties, because the great ability of halophilic yeast to promote growth and germination in high soil salinity makes it very important in meeting the challenges that the agricultural sector will face in the future, thus offering a sustainable way to increase crop production to meet the ever-growing demand for food (Lafitte et al., 2004).

This work is a little example of how the agroecological approach that promotes interaction between organisms and different trophic levels is more sustainable and more suitable to face all the modern agricultural challenges.

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Assessment of the mitigating capacity of saline stress by NK fertilizer

Abstract

The use of syntethic fertilizers has led to a massive increase in crop yields, but the overuse of chemical N compounds has led to massive pollution of water, air and, above all, soil, especially secondary soil salinization. However, it suggested that organic fertilizer has the capability to reduce abiotic stresses and enhance crop production. The aim of this study is to evaluate the salt and heat stress mitigating capability of NK fertilizer (KNO_3). The study focused on 2 ancient varieties ("Turanico" *Triticum turanicum* Jacubz., 1947 and "Einkorn" *T. monococcum* L.) and one modern crop (*T. durum* Desf var. Claudio). To test salt stress mitigating capacity germination tests were carried out 100, 200 and 300 mM of NaCl. To evaluate salt - temperature interaction, germination tests has been conducted at 20, 25, 30 °C. The varieties showed significantly different sensitivity to fertilizer mitigating capability. Einkorn due to its marked stress tolerance showed a fertilizer benefit, by 5-10%. Instead, Turanico and Claudio germination has been enhanced on average by 20 and 30 % respectively.

Despite the enhanced germination due to the fertilizer effect, Einkorn and Turanico showed a remarkable resistance to salt stress. This part of the project shows that fertilization, can be a way to mitigate climate change and ensure future food security.

Introduction

With the onset of the green revolution and the advent of industrial agriculture, natural fertilizers derived from complementary activities to cultivation were replaced by highly effective chemical compounds (Altieri, 2009). The use of artificial fertilizers in conjunction with the use of high-yielding varieties has led to a doubling of yields in cereal varieties. In fact, average production has increased from about 30 quintals per hectare to 70 quintals per hectare (Vermeulen at al., 2012). However, the unbridled use of chemical fertilizers has many negative consequences. One of the biggest problems associated with fertilizer abuse is the pollution of groundwater (Khan et al., 2018). Synthetic fertilizers such as ammonium nitrate easily decompose into nitrates and can cause bioaccumulation and biomagnification phenomena (Khan et al., 2018).

Recent studies have linked water contamination to various cancers, fetal malformations and methaemoglobinaemia (Fewtrell,2004). Groundwater fertilizer contamination also leads to dead zones in the sea, as excess nutrients cause an excessive algal growth with massive oxygen depletion and widespread mortality of fish and crustaceans (Khan et al., 2018).

In the soil, the application of synthetic nitrogen leads to a reduction in organic matter. This process works through a feedback mechanism, i.e. as the organic matter is reduced, the soil's ability to bind it decreases. The reduction of organic matter in the soil also leads to compaction problems and increases the risk of runoff, erosion and greater difficulty in root growth (Khan et al., 2018). Damaged soils become compacted, making them susceptible to runoff, erosion, lower water holding capacity and severely restricting plant root growth. In addition, one of the most important harmful effects of the use of artificial fertilisers is the *secondary* salinization of soils (Khan et al., 2018).

High salinized soils could thus induce morphological, physiological, and biochemical changes in plants, resulting in a stunted growth and crop nutritional value reduction (Akbari et al., 2020; Naifer et al., 2011). Salinity can also decrease the germination percentage and increase germination time by delaying the emergence, with a general decreased of germination at increasing salt concentration start (Del Vecchio et al., 2018; Muñoz-Rodríguez et al., 2017). These harmful phenomena are further exacerbated by the ongoing climate changes, specifically by the increase of thermal regimes.

The use of organic fertilizers may represent an effective approach to enhance crop tolerance to salt stress and uplift global crop cultivation under stressful environment (Hussein and Abou-Baker 2018; Adisa et al. 2019). In organic fertilizer lies the ability to generate the biochemical processes responsible for the absorption and translocation of other elements and consequently lead to an improvement in the balance of nutrients and thus to better growth. (Sassine et al., 2020). For instance, the application of manure significantly increased growth and tolerance of tomato plants (*Solanum lycopersicum* L.) in salt stress conditions (Sassine et al., 2020). Besides mitigating salt stress, the application of organic fertilizer may contribute to improve soil quality, according to Zhang et al. (2020), manure exploitation leads to increased soil carbon and reduced atmospheric carbon levels, reduced soil erosion and runoff, reduced nitrate leaching, and reduced demands for intensive nitrogen (N) fertilizers (Zhang et al., 2020). Manure organic matter also contributes to improve soil structure, resulting in more efficient water infiltration and greater water-holding capacity that, in turn, lead to a decreased crop water stress, soil erosion, and increased nutrient retention (Meng et al., 2005). Application of low salt index N compounds can be thus a comprehensive and suitable choice to solve problems related to human and plant health, livestock welfare and sustainable environment.

In this scenario, the enhancement of agrobiodiversity and the exploitation of stress tolerant crops, i.e., traditional cereal varieties, may represent a viable route to mitigate soil salinization .

Traditional cereal varieties are an outstanding example of stress tolerant crops (Maucieri et al., 2018). They have been proved to have a great resistance to environmental stress, superior nutritional content, and great adaptability due to their higher genetic diversity (Casañas et al., 2017). However, their use has been decreasing mostly due to their lower productivity compared to modern varieties (Moudry et al., 2012; Casañas et al., 2017).

In light of these, the aim of this work was to verify whether the application of organic fertilizer may contribute to increase landrace salt tolerance. To this aim, we tested the effect of NPK fertilizer on the germination phase of crop life cycle, since the germination phase has been recognized as a reliable and rapid method to evaluate crops stress tolerance (Ashraf et al., 1987).

Method

To test and compare salt stress mitigation of organic fertilizer, we selected seeds of two traditional varieties, i.e., "Turanico", *Triticum turanicum* Jacubz., 1947, "Einkorn" *T. monococcum* L., and one modern variety, *T. durum* Desf. var. Claudio. Seeds were obtained from the Rural Seed Network of Umbria. All the selected varieties were harvested in July 2018 and then placed in the storage cell (18 °C, 40% humidity). The chosen three cereal varieties were briefly descripted in Table 1.

Table 12 Description of the tested varieties (De Cillis, 1927; Trebbi et al., 2011)

Variety	Species	Registration Year	Country	Pedigree
Einkorn	*Triticum monococcum L.*	/	Italy	/
Turanico	*Triticum turanicum Jakubz., 1947*	1921	Italy	/
Claudio	*Triticum durum Desf.*	1999	Italy	CIMMYT35/Durango//IS1938/Grazia

Germination Tests

The viability of the seeds and their sterilisation before the germination tests were carried out as described above .

For each crop variety and condition, 10 replicates of 20 seeds each were sown in a sterile environment on two sheets of Whatman No.1 filter paper in 9-cm diameter Petri dishes.

To assess salt stress mitigation effect of organic fertilizer, seeds were exposed to 100, 200, and 300 mM of NaCl; for each salt condition we tested 3 fertilizer concentration; specifically, 15 kg/ha (N1), 25 kg/ha (N2) and 35 kg/ha (N3) of KNO_3. These values have been chosen because they are the quantity of nitrogen parts used in the pre-sowing ground fertilization (Amicabile, 2016). Moreover, to identify thermal influence, seeds were incubated into a growth chamber (Vetrotecnica UW134 AT1.69 CAJ4492Y Opn pXS HG TCRH2SS EV Spec) at 20 ± 1,5°C (thermal optimum) (Orsenigo et al., 2017), 25 ± 1,5°C and 30 ± 1,5°C, for 30 days. The scoring has been carried out in a laminar flow hood to minimize microbiological contamination (Baskin and Baskin, 2014).

Data analysis

To analyse the mitigation effect of organic fertilizer and the interaction effect of fertilizer on the germination of the three crop varieties under salt and heat stress, we calculated three parameters (Joosen et al., 2010), averaged by the 10 replicates for each tested condition: a) germination percentage, calculated as the number of germinated seeds on the total number of sown seeds, multiplied by one hundred; b) median germination time (T50), namely the time (in days) required to achieve 50% of germination; c) germination delay, as the number of days until the first germination occurred.

$$T50 = t1 + \frac{\left[\left(\frac{N}{2} - n1\right)(t1 - t2)\right]}{n1 - n2}$$

For each abiotic conditions, germination percentage, T50 and germination delay were compared by one-way PERMANOVA test with 999 randomizations, using salt and heat stress as grouping variables. Fisher's least significant difference (LSD) was then performed to identify significant differences of the calculated parameters among varieties.

Results

Salt presence significantly (PERMANOVA test; $p <0.05$; Table 2) affected germination parameters (germination percentage, of all the tested varieties. However, the tested crops were differently affected by salinity stress; Claudio is the most sensitive variety, because at 20° C and 300 mM a germination of 39.56 ± 1.95 per cent is observed. Conversely, Turanico and Einkorn showed a marked salt tolerance, with a percentage of germination that is attested of 72.87 ± 1.50, averaged between the two varieties. The germination of all the varieties has been significantly affected (LSD test; $p < 0.05$, Table 2) by the temperature of 25 and 30°C. The germination test showed a heterogeneous response to the heat stress, indeed Einkorn and Turanico are characterized by a greater heat tolerance compared to the modern varieties Claudio. The test showed that the interaction between salt and high temperature had a synergistic detrimental effect on seeds germination. Indeed at 30 °C and 300 mM NaCl, Claudio, Turanico and Einkorn germination rate steeply decreased to 1.75% ± 0.87, 0 and 48.2 ± 2.4 respectively (Table 4).

The exposure of seeds to fertilizer led to a heterogeneous response pattern among the varieties. Einkorn showed no significant (Table 2 $p>0.05$) mitigating effect when tests were conducted at 20°C. However, at 25°C fertilizer had the capability of increase the germination by 10 and 5 % respectively for 200 and 300 mM. Furthermore, NK compounds enhanced by 30% seed germination at the thermal regime of 30°C (Table 4).

Conversely, fertilizer interaction with Turanico led to a different and particular germination behaviour related to temperature. At 20°C low concentration of KNO_3 (N1 and N2) did not have a mitigating effect on salt stress, however N3 of fertilizer significantly increase germination rate about of 30 % when seeds were treated with 300 mM of NaCl. N1 and N2 concentration of fertilizer demonstrated the capability of increasing germination rate by 20% when salt stress tests were conducted at 25 °C. However, at 30°C NK fertilizer showed no mitigation of salt stress, furthermore KNO_3 could have detrimental effect in high stress conditions, indeed the interaction among N3 concentration and 300 mM of NaCl led to seeds germination of 0.

The modern variety Claudio showed the expected pattern of response. When seeds were exposed to 25 and 30 °C in all salt concentration NK fertilizer led to a significant increase (PERMANOVA, $p<0.05$, Table 2 and Table 4) of the germination rate. At 25 ° C, an average increase in germination of 20% was observed with the application of N1 and N2 of KNO3, moreover, at 30 ° C, the N1 and N2 fertiliser resulted in a 30% increase in the germination of seeds under salt stress. On the other hand, the total fertiliser levels at 20°C did not show any significant dampening effect. Unfortunately, the germination delay and the medium germination time resulted biased because of the aperiodic controls of the germinated seeds done during the Covid-19 pandemic. This circumstance led to the exclusion of these data that would not be scientifically reliable.

Table 2 PERMANOVA results of the germination percentage at different salt, fertilizer concentration and temperature for the tested varieties.

		Germination Percentage		
		DF	F Model	P-value
Claudio	Salt	1	62.433	0.001
	Temperature	1	18.916	0.001
	Fertilizer	1	18.916	0.001
	Salt x Fertilizer	1	41.93	0.001
	Temperature x Fertilizer	1	7.283	0.001
T.turanicum	Salt	1	62.857	0.001
	Temperature	1	19.044	0.001
	Fertilizer	1	42.214	0.001
	Salt x Fertilizer	1	8.299	0.001
	Temperature x Fertilizer	1	10.455	0.001
Einkorn	Salt	1	42.441	0.001
	Temperature	1	12.984	0.001
	Fertilizer	1	28.787	0.001
	Salt x Fertilizer	1	5.659	0.001
	Temperature x Fertilizer	1	7.128	0.001
	Residuals	202		

Table 3 PERMANOVA results of the germination percentage at different salt, fertilizer concentration and temperature among the tested varieties.

	Germination Percentage		
	DF	F Model	P-value
Variety	3	101.258	0.001
Salt	1	21.033	0.001
Temperature	1	67.626	0.001
Fertilizer	1	4.342	0.001
Salt x Fertilizer	2	24.947	0.001
Salt x Temperature	2	20.018	0.001
Variety x Salt	2	2.986	0.001
Variety x Fertilizer	2	2.645	0.001
Variety x Temperature	2	31.703	0.001
Residuals	599		

Table 4 Means ± SD for germination percentages. Values followed by different capital letters significantly differ among fertilizer concentration. Values followed by small letters significantly differ within each salt concentration (Fisher LSD) test.

Turanico

Fert	20 °C				25 °C				30 °C			
	0	100 mM	200 mM	300 mM	0	100 mM	200 mM	300 mM	0	100 mM	200 mM	300 mM
c	97.5± 6.34Aa	92.5± 5.94Aa	86.2±3.8Ab	67.5± 7.14Ac	98.75±4.93Aa	72.5±3.62Ab	71.25±3.56Ab	87.5±4.375Ac	85±4.25Aa	62.5±3.125Ab	7.5±0.37Ac	0±0Ad
N1	93.75±2.4Aa	82.5± 5.77Bb	85± 3.33Ab	73.75± 5.8Ac	95±4.75Aa	91.25±4.56Bb	87.5±4.37Bb	70±3.5Bc	62.5±3.125Ba	46.25±2.3125Bb	10±0.5Ac	3.75±0.18Bd
N2	95± 2.5Aa	86.25±8.6Cb	81.2±2.5Ac	76.25± 2.4Ad	97.5±4.87Aa	90±4.5Ab	78.75±3.93Cc	78.75±3.93Cc	73.75±3.6875Ca	26.25±1.3125Cb	2.5±0.12Bc	3.75±0.18Bc
N3	96.25±4.8Aa	88.75±8.8Cb	77.5±5.5Ac	92.5± 4.67Bd	92.5±4.62Aa	85±4.25Bb	71.25±3.56Ac	67.5±3.37Dc	52.5±2.625Da	33.75±1.6875Db	5±0.25Cc	0±0Ad

Einkorn

Fert	20 °C				25 °C				30 °C			
KNO_3	0	100 mM	200 mM	300 mM	0	100 mM	200 mM	300 mM	0	100 mM	200 mM	300 mM
c	99±4.95Aa	98.25±4.9Aa	92.8±4.6Aa	78.25±3.91Ab	95.7±4.79Aa	95±4.75Aa	80±4Ab	72±3.6Ac	96.25±4.81Aa	58±2.9Ab	51±2.55Ac	48±2.4Ac
N1	97.5±4.88Aa	95±4.75Aa	75±3.7Bb	73.75±3.69Ab	97.5±4.88Aa	98.75±4.94Ba	83.75±4.19Ab	76.25±3.8Ac	90±4.5Ba	85±4.25Bb	58.7±2.9Bc	32.5±1.63Bd
N2	100±5Aa	97.5±4.88Aa	88.7±4.4Cb	86.25±4.31Bb	93.75±4.7Ba	98.75±4.94Ba	92.5±4.63Bb	73.75±3.69Ac	92.5±4.6Ca	81.25±4.06Cb	42.5±2.1Cc	32.5±1.63Bd
N3	96.25±4.8Aa	88.75±4.4Bb	85±4.25Cb	78.75±3.94Cc	95±4.75Aa	90±4.5Cb	86.25±4.31Cb	61.25±3.06Bc	92.5±4.63Ca	76.25±3.81Db	43.7±2.1Cc	53.75±2.6Cd

Claudio

	20 °C				25 °C				30 °C			
Fert	0	100 mM	200 mM	300 mM	0	100 mM	200 mM	300 mM	0	100 mM	200 mM	300 mM
c	92.25±4.61Aa	85±4.25Ab	88±4.4Ab	39±1.95Ac	83.75±4.19Aa	57.5±2.88bA	53±2.65Ab	33.75±1.69Ac	77.5±3.88Aa	33.25±1.66Ab	39.25±1.9Ab	1.75±0.09Ac
N1	76.25±3.81Ba	73.75±3.69Bb	71.2±3.5Bb	80±4Bc	86.25±4.31Aa	80±4Bb	70±3.5Bc	42.5±2.13Bd	81.25±4.06Ab	75±3.75Bb	62.5±3.13Bc	67.5±3.38Bc
N2	88.75±4.44Ca	71.25±3.56Bb	71.2±3.5Bb	75±3.75Bb	80±4Ba	76.25±3.81Bb	73.75±3.69Bc	71.25±3.56Cc	67.5±3.38Ba	67.5±3.38Ca	53.75±2.6Cb	38.75±1.9Cc
N3	70±3.5Da	83.75±4.19Cb	61.2±3.0Cc	55±2.75Cd	83.75±4.19Aa	66.25±3.31Cb	65±3.25Cb	76.25±3.81Cc	65±3.25Ba	68.75±3.44Ca	42.5±2.13Db	46.25±2.3Cc

Discussion

Our results show that NK fertiliser treatment may be a viable option to salt stress. Our results are in agreement with those of Ali et al. (2021), who found that nitrogen effectively protected the early growth stage of sorghum seedlings from damage caused by salt stress, and Ibrahim et al. (2016) also demonstrated a reduction in salt stress in wheat seedlings. but the tested varieties reacted with a heterogeneous pattern. Einkorn proved to be among the tested varieties the most resistant to salt and heat stress, indeed this varieties showed an outstanding seeds germination at 30°C and 300 mM, abiotic condition where most of crops failed to germinate. However, despite this exceptional resistance, exposure of Einkorn seeds to the N1 concentration resulted in a significant increase in germination rate of 5 % and 10 % at 25 °C with 200 and 300 mM and 30 % at 30 °C and 100 mM of NaCl. Instead, Turanico demonstrated to have a medium high salt tolerance, but is more vulnerable to heat stress, indeed at 30°C and 300 mM of NaCl seeds vitality is 0. As already mentioned for einkorn, also for Turanico NK treatment led to a mitigation of salt and heat stress.

The modern variety Claudio is the most sensitive to abiotic stresses, in fact seeds germination steeply decreased already at low-medium stress level, however an outstanding germination increase occurred due to the interaction with KNO_3.

The greater tolerance of Einkorn and Turanico may be explained by a higher capacity of water absorption at the beginning of the germination process, which allows stable germination percentage even at the highest salt and heat stress (Maucieri et al., 2018). According to Casanas et al. (2017), the greater abiotic resistance of traditional varieties compared to the modern ones may also reside in their genetical heterogeneity and the absence of crop improvement and homogenization.

However, it is important to highlight the phylogenetic link between Turanico and Claudio (Petersen et al., 2006). Indeed, from the results of the germination percentage, it appears that Turanico is more sensitive than einkorn and has a closer phenotypic relationship with Claudio. Einkorn and Turanico treated with NK treatment under salt and heat stress led to a peculiar response pattern, occurred in both varieties. At relative low stress levels (salt and heat cumulated) fertilizer had no significant effect on the germination percentage, however as stress level increased, a physiological switch occurred, in which the NK compounds acquired the capability of mitigating abiotic stress. The stress level of this switch seems to be strictly species-specific and could be related to the inherit variety tolerance, i.e., einkorn has this physiological switch at 25°C and 200 mM of NaCl, instead in the Turanico this phenomenon occurs at lower stress level (20°C and 300 mM of NaCl). This hypothesis is consistent with the existing literature; Maggio et al. (2007) observed a similar stress response pattern in *Lycopersicon esculentum* Mill. This physiological switch could be due to ABA (abscisic acid) that triggers a transition from molecular/cellular adaptation to more complex structural/morphological modifications (Maggio et al., 2007).

Findings consistent with this work were observed in cabbage, in which Xin et al. (2017) reported a reduction of adverse effects of various stresses after manure administration by influencing inorganic nutrient uptake and accumulation.

In addition, organic N (manure N tied to organic compounds) is more stable than N applied as commercial fertilizer. Conventional ammonium nitrate is soluble in water and mobile, contributing to leaching during excess precipitation (e.g., spring rains prior to or early in growing season) or irrigation (Amicabile, 2016). Manure N's slow transformation to nitrate is better timed to crop N needs, resulting in less leaching potential. In fact, manure N is a natural slow-release form of N (Amicabile, 2016). Moreover, substituting manure for chemical fertilizers will reduce crop production energy costs and the environmental impact of production chain (Bentrup et al., 2016).

Einkorn and Turanico wheat proved to have great stress tolerance without N enhancement, however this work indicates a viable tool in mitigating salt stresses and reduce pollution.

Further studies need to be carried out to test and assess other organic fertilizer – varieties interaction in order to exploit their outstanding properties in mitigating climate change and restoring soil properties.

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Evaluation of the productivity of traditional varieties

Abstract

Global consumption of cereals is projected to increase by 1.7% to 2.8 million tons in 2021/22, reaching 826 million tons. Total consumption of cereal-based foods will increase in step with the world's population, while the use of cereals for animal feed is also expected to rise. Despite rising production, cereals yield is currently insufficient to meet global food needs, and will hardly be so in the future, indeed climate change will lead to a drastic decline in cereal production by 2100, ranging from 17 to 33%. The aim of this part of the project is to evaluate the productivity of traditional varieties in terms of vegetative and grain growth using the organic and conventional methods, also to identify varieties with optimal agronomic traits for breeding resistant and productive varieties. The following traditional varieties were sown: - "Emmer" *Triticum dicoccum* L., "Einkorn" *Triticum monococcum* L., *Triticum durum* Desf. var Senatore Cappelli, *Triticum durum* Desf. var Timilia, *Triticum aestivum* L. var. Verna and the following modern variety: *Triticum durum* Desf. var. Claudio. Claudio showed to have a higher productivity at the seed level, however, traditional varieties have a more pronounced and greater vegetative growth than modern varieties. This stronger vegetative growth is reflected in a greater height, Tillage Index and SLA and resistance to pathogens in the traditional varieties than in Claudio.

Introduction

The UN Agenda for Sustainable Development is a set of 17 interrelated goals defined by the United Nations as a strategy "to achieve a better and more sustainable future for all"(UN, 2015). One of the most important Sustainable Development Goals is number 2, i.e. "End hunger, achieve food security and improved nutrition, and promote sustainable agriculture". In concrete terms, this goal is primarily about providing the entire world population with sufficient and high-quality food (UN, 2015). The world's dietary habits consist mainly of the consumption of cereals, especially rice, maize and wheat. Global consumption of cereals is projected to increase by 1.7% to 2.8 million tons in 2021/22, reaching 826 million tons. Total consumption of cereal-based foods will increase in step with the world's population, while the use of cereals for animal feed is also expected to rise (FAO, 2020). Despite rising production, cereals yield is currently insufficient to meet global food needs, and will hardly be so in the future, unless major changes in the production paradigm, due to the seamless increase in world population - by 2050 the world population will be 10 billion - and especially due to climate change (FAO, 2020). According to Gammans et al. (2017), climate change will lead to a drastic decline in cereal production by 2100, ranging from 17 to 33%. This sharp decline will be caused mainly by a rise in temperatures combined with a shift in precipitation regimes, with limited periods of intense rainfall alternating with increasingly long dry periods, leading to an increase in drought and soil salinization. Warmer temperatures affect crops in a number of ways, including poor plant growth, reduced photosynthesis, leaf senescence, reduced pollen viability and consequently the production of fewer grains with smaller grain size (McClung and Davis 2010; Grant et al. 2011). Salt and drought stress can lead to changes in gene expression that are important for plant life support (Batlang et al. 2013), reduced protein production that can damage crops (Mohammadi et al. 2012), and changes in metabolic activity that affect crop production (Kumari et al. 2016). A further consequence of climate change is the emergence and development of pathogens and weeds that are becoming increasingly aggressive and resistant to herbicides and pesticides, which will have a significant impact on the production of arable land in the near future (Altieri et al., 2009). This vulnerability of the modern agricultural system, especially grain production, to climate change is due to the Green Revolution, the phenomenon that led to a profound intensification, industrialization and mechanization of agriculture. In particular, there has been an over-exploitation of external inputs (pesticides and fertilizers), but above all a massive use of hyper-productive wheat varieties characterized by high genetic homogeneity (Kahane et al., 2013). According to Altieri et al. (2009), high genetic variability within and between species is one of the most important characteristics that an agroecosystem must have in order to promote sustainable development and resist climate change. Di Falco and Perrings (2005), who studied different cereal farms in southern Italy, have shown that increasing spatial plant diversity can mitigate climate change negative effects. Evidences shows how increased diversity supports the resilience of agroecosystems and promotes productivity when physical factors become a constraint.

One way of adapting the system of cereal production may therefore be to reintroduce traditional cereal varieties. In other words, all those cereal varieties that have undergone little or no genetic improvement and locally adapted by the farmers and indigenous local cultures (De Cillis, 1927).

These cereal varieties are characterized by greater genetic variability, which makes them more resistant to abiotic and pathogenic stresses, and most importantly, they require reduced or no external inputs for cultivation. In addition, these varieties have a better nutrient and antioxidant profile due to less genetic improvement and, above all, better digestibility due to a lower gluten content (Ghiselli et al., 2016). However, they are characterized by lower productivity in tons of wheat than modern varieties, in marginal or stressed areas, however, this difference in production is reduced to zero (Casanas et al., 2017). In view of their remarkable properties, the reintroduction of organic cultivation of these varieties is currently underway. Organic farming is ideal because it makes the most of the characteristics of traditional varieties, as this method aims to produce high quality food and minimize farming practices and the use of specific external inputs (Amicabile, 2016). Due to climate change, more and more adapted and productive varieties are being created. To this end, traditional varieties could be used as initial varieties for the creation of new highly productive and resistant crops through genetic improvement. (Ceccarelli. 2016). The aim of this part of the project is to evaluate the productivity of traditional varieties in terms of vegetative and grain growth using the organic and conventional methods, also to identify varieties with optimal agronomic traits for breeding resistant and productive varieties.

Methods

Cultivation

The two-season field trial was conducted in the winter wheat seasons of 2018-2019 and 2020-2021 at the experimental station of the La Battistei Farm, located in North Italy (Cittadella Municipality 45.674 N, 11.737 E and 70 m elevation). The average annual rainfall from 1951 to 2014 at the station was 1429 mm, the average seasonal air temperature was 13.0 °C, and the average sunshine duration was 2978.72 h. The soil was ploughed to a depth of 20 cm with a tractor-drawn disc plough and large clods of soil were smoothed with a harrow to create a level seedbed. In both years the following traditional varieties were sown: - "Emmer" *Triticum dicoccum* L., "Einkorn" *Triticum monococcum* L., *Triticum durum* Desf. var Senatore Cappelli, *Triticum durum* Desf. var Timilia, *Triticum aestivum* L. var. Verna and the following modern variety: *Triticum durum* Desf. var. Claudio, a more detailed description is given in Table 1.

Table 13 Description of the tested varieties (De Cillis, 1927; Ghiselli et al., 2016; Trebbi et al., 2011)

Variety	Species	Registration Year	Country	Pedigree
Verna	*Triticum aestivum* L.	1953	Italy	Est Mottin/Mont Calme 245
Einkorn	*Triticum monococcum L.*	/	Italy	/
Emmer	*Triticum dicoccum L.*	/	Italy	/
Senatore Cappelli	*Triticum durum Desf.*	1930	Italy	Selection from Jennah Rhetifa
Timilia	*Triticum durum Desf.*	/	Italy	/
Claudio	*Triticum durum Desf.*	1999	Italy	CIMMYT35/Durango//IS1938/Grazia

The sowings were carried out on 25 October 2018 and 11-12 November 2020. The identified varieties were sown with double replication in plots of 5 m2 for both conventional and organic methods. 250 and 400 seeds per mm^2 were sown for the traditional and modern varieties, respectively. During the growing season, 30 g of ammonium nitrate (26% N) for Claudio and 15 g for the traditional varieties were applied to the varieties grown using the conventional method, particularly on 18 February 2019, 11 March 2020 and 23 April 2020 to support growth. However, on the same days, 60 g of Super Robur (15%) for Claudio and 30 g for the traditional varieties were applied to the organically grown varieties. Nitrogen fertiliser, however, was not administered to both einkorn and emmer, as it is not necessary for cultivation, but on the contrary increases the tendency to lodging. To prevent fungal infections, the varieties were sprayed with tetraconazole on 18 March 2019 and 11 March 2021 for the organically grown varieties and organic fungicide for the organic test. The conventional method tests used a dicotyledonous herbicide for seasonal weed control, while the biological tests used mechanical weed control. The harvest took place on 1 July 2019 and 23 June 2021.

Statistical Analysis

In each production method, each variety was grown in duplicate in 2 plots of 3.75 m^2, from which 10 individuals were randomly selected, on which measurements were made during the season to characterize them morphologically and agronomically; for each variety grown, the following parameters were measured Height (cm), Tillering index : specifically number of fertile shoots per plant, , Seeds per spike, Percentage of infected seeds, number of infected seeds out of 100 seeds examined, Weight 100 seeds (g), Ratio seed biomass / vegetative biomass, SLA (mm^2 / mg) (Cornelissen et al., 2003; Amicabile, 2016) . The specific leaf area (SLA) is a ratio that indicates how much leaf area a plant forms with a given amount of leaf biomass. To calculate the SLA, 4 green leaves were collected for each selected individual in the period before flowering, the fresh weight was calculated and the leaf area was determined using the Leaf Area programme. The leaves were then dried at 80° C for 48 hours and weighed again to obtain the dry weight (Cornelissen et al., 2003). For each variety, all specified parameters were compared by a one-sided ANOVA test with 999 randomizations, using the varieties as grouping variables. Fisher's test (LSD) was then performed to detect significant differences in the calculated parameters between the varieties.

Results

The results of our investigation are consistent with the bibliography and the assumptions made earlier. A look at Tables 2 and 3 shows that the modern variety Claudio has a higher productivity at the seed level, namely an average number of seeds per ear of 40 in the first year and 51 in the second year of cultivation, compared to an average of 27.7 in the first year and 33 in the second year of the traditional varieties. Moreover, the hectoliter weight of the modern variety Claudio, 4.8 g and 5.37 g in the second year, is higher than that of the traditional varieties, which have an average hectoliter weight of 4.1 g in the first and 3.9 g in the second year of cultivation. However, the Senatore Cappelli variety is characterized by a higher hectoliter weight than the modern Claudio variety. Despite the lower productivity in terms of seeds, the traditional varieties show greater vegetative growth, in particular a greater average height is observed h = 129.5 cm in the first year and 139.05 cm in the second year of the traditional varieties compared to 88 and 72 cm in the first and second years of Claudio. In particular, an average height of 154.1 cm was observed in einkorn in the second year. All other vegetative parameters observed (Tillering Index, SLA) were higher in the traditional varieties than in Claudio. A very important parameter to evaluate is the ratio between seed biomass and plant biomass, which indicates the strategy of resource allocation. In Claudio, a very high ratio is observed, indicating a strategy oriented towards seed production, while in Einkorn the ratio is very low, indicating pronounced vegetative growth. Instead, there is a higher ratio of seed biomass to vegetative growth in both Timilia, Verna and Senatore Cappelli, indicating a more balanced strategy of resource allocation between vegetative growth and seed production. One of the most important parameters is the calculation of the percentage of seeds infected with Fusarium or Puccinia, which is used as a proxy for calculating resistance to pathogens. In both crop years, the Claudio variety has a much higher percentage of infections (33% and 25%) compared to the traditional varieties. In particular, it is observed that Einkorn, Timilia and Verna have a very low percentage of infections, even under conditions favorable to the occurrence of fungi. As can be seen from Tables 2 and 3, no significant differences were found between the conventional and organic production methods

Table 2 Table with the parameters measured in the first year of cultivation, values followed by different capital letters differ between varieties, values followed by different lowercase letters differ between production methods.

	Height		Tillering Index		Seeds per Spike		% of Infected Seeds	
	Conv	Organic	Conv	Organic	Conv	Organic	Conv	Organic
Claudio	88±4.4 Aa	84.48±4.22 Aa	1.12±0.06 Aa	1.0976±0.05 Aa	40.2±2.01 AAa	38.19±1.91Aa	29±1.45Aa	28.42±1.42Aa
Einkorn	154.1±7.71Ba	147.936±7.4Ba	1.85±0.09Ba	1.813±0.09Ba	27.2±1.36Ba	25.84±1.29Ba	4±0.2Ba	3.92±0.2Ba
Emmer	138.5±6.93Ca	132.96±6.65Ca	1.5±0.08Ca	1.47±0.07Ca	27.7±1.39Ca	26.315±1.32Ca	9±0.45Ca	8.82±0.44Ca
Senatore Cappelli	135.5±6.78Da	130.08±6.5Da	1.2±0.06Da	1.176±0.06Da	28.6±1.43Da	27.17±1.36Da	22±1.1Da	21.56±1.08Da
Verna	140.2±7.01Ea	134.592±6.73Ea	1.6±0.08Ea	1.568±0.08Ea	33.7±1.69Ea	32.015±1.6Ea	7±0.35Ea	6.86±0.34Ea
Timilia	141.36±7.068Fa	135.7056±6.78Fa	1.67±0.083Fa	1.6366±0.081Fa	26.4±1.32Fa	25.08±1.25Fa	6±0.3Fa	5.88±0.294Fa

	Weight 100 seeds(g)		Seed /Vegetative biomass		SLA	
	Conv	Organic	Conv	Organic	Conv	Organic
Claudio	4.8±0.24Aa	4.536±0.23Aa	0.13±0.01Aa	0.1261±0.01Aa	16.61±0.83Aa	16.4439±0.82Aa
Einkorn	3.3±0.17Ba	3.1185±0.16Ba	0.07±0.003Ba	0.0679±0.004Ba	25.41±1.27Ba	25.1559±1.26Ba
Emmer	4.2±0.21Ca	3.969±0.2Ca	0.12±0.01Ca	0.1164±0.01Ca	20.88±1.04Ca	20.6712±1.03Ca
Senatore Cappelli	5.8±0.29Da	5.481±0.27Da	0.14±0.01Da	0.1358±0.01Da	29.087±1.45Da	28.79613±1.44Da
Verna	4.4±0.22Ea	4.158±0.21Ea	0.083±0.08Ea	0.08051±0Ea	21.64±1.08Ea	21.4236±1.07Ea
Timilia	4.5±0.225Fa	4.2525±0.21Fa	0.084±0.0042Fa	0.08148±0.0040Fa	24.62±1.23Fa	24.3738±1.21Fa

Table 3 Table with the parameters measured in the second year of cultivation, values followed by different capital letters differ between varieties, values followed by different lowercase letters differ between production methods.

	Height		Tillering Index		Seeds per Spike		% of Infected Seeds	
	Conv	Organic	Conv	Organic	Conv	Organic	Conv	Organic
Claudio	72.65±3.633Aa	71.93±3.597Aa	3.8±0.19Aa	3.65±0.183Aa	51.12±2.556Aa	50.6±2.53Aa	23±1.15Aa	22.54±1.127Aa
Einkorn	144.5±7.225Ba	143.06±7.153Ba	6.1±0.305Ba	5.86±0.293Ba	27.7±1.385Ba	27.42±1.371Ba	3.5±0.175Ba	3.43±0.172Ba
Emmer	142.81±7.141Ca	141.38±7.069Ca	5.5±0.275Ca	5.28±0.264Ca	31.96±1.598Ca	31.64±1.582Ca	4.32±0.216Ca	4.23±0.212Ca
Senatore Cappelli	128.1±6.405Da	126.82±6.341Da	5.4±0.27Da	5.18±0.259Da	36.88±1.844Da	36.51±1.826Da	15.64±0.782Da	15.32±0.766Da
Verna	139.89±6.995Ea	138.49±6.925Ea	4.86±0.243Ea	4.66±0.233Ea	33.36±1.668Ea	33.03±1.652Ea	5.45±0.273Ea	5.35±0.268Ea
Timilia	129.03±6.452Fa	127.74±6.387Fa	5.25±0.263Fa	5.04±0.252Fa	35.12±1.756Fa	34.77±1.739Fa	9.45±0.473Fa	9.26±0.463Fa

	Weight 100 seeds(g)		Seed /Vegetative biomass		SLA	
	Conv	Organic	Conv	Organic	Conv	Organic
Claudio	5.37±0.269Aa	5.32±0.266Aa	0.18±0.009Aa	0.18±0.009Aa	17.34±0.867Aa	16.99±0.85Aa
Einkorn	3.5±0.175Ba	3.47±0.174Ba	0.08±0.004Ba	0.08±0.004Ba	29.56±1.478Ba	28.97±1.449Ba
Emmer	5.53±0.277Ca	5.47±0.274Ca	0.1±0.005Ca	0.09±0.005Ca	31.57±1.579Ca	30.94±1.547Ca
Senatore Cappelli	6.36±0.318Da	6.3±0.315Da	0.12±0.006Da	0.12±0.006Da	23.45±1.173Da	22.98±1.149Da
Verna	4.48±0.224Ea	4.44±0.222Ea	0.08±0.004Ea	0.08±0.004Ea	27.66±1.383Ea	27.1±1.355Ea
Timilia	2.35±0.118Fa	2.33±0.117Fa	0.05±0.003Fa	0.04±0.002Fa	33.27±1.664Fa	32.6±1.63Fa

Discussion

This part of the project has shown that the modern variety Claudio has a higher yield at seed level than traditional varieties. In fact, the Claudio variety has a larger number of seeds and a higher hectoliter weight compared to the traditional varieties. However, within the group of traditional varieties, we found some variability. In fact, Senatore Cappelli has a higher productivity than the other tested varieties in terms of number and weight of seeds. This higher productivity of modern varieties is due to the genetic improvement of modern varieties, which has led to the creation of very productive varieties in terms of seed and with reduced vegetative growth. In addition, genetic improvement has led to extreme genotypic and phenotypic homogenization, to enable ever more advanced automation and mechanization with ever higher yields and efficiencies (Amicabile, 2016). Despite lower productivity at the seed level, traditional varieties have a more pronounced and greater vegetative growth than modern varieties This stronger vegetative growth is reflected in a greater height, Tillage Index and SLA in the traditional varieties than in Claudio. In addition, traditional varieties have a lower seed biomass to vegetative ratio, indicating a rice allocation strategy that is more focused on vegetative growth.

This pronounced growth in the early 1900s was chosen to allow wheat to be grown even in slightly swampy or flood-prone areas, but above all to compete with weeds without the addition of herbicides (De Cillis, 1927). However, this vegetative growth was one of the traits to be eliminated by genetic improvement, because taller varieties are very susceptible to lodging. Moreover, varieties with a high tillage index require a lower sowing density to avoid water stagnation phenomena and to favor lodging (Amicabile, 2016). However, this increased vegetative growth can be an exceptional resource for the forage industry. Indeed, the entire expanding livestock sector will require varieties with high vegetative growth, high biomass and a high nutritional index for the production of straw and hay (Ghiselli et al., 2016). In terms of production at both seed and vegetative levels, no significant difference was found between the organic and conventional methods for the traditional varieties. This is another advantage of their use, as the yield of the varieties did not decrease due to a more conservative production method and with a lower contribution of external inputs and agricultural practices.

Comparing the vegetative and reproductive growth parameters of the traditional varieties in the 2 different growing seasons, a marked stability was observed. This phenomenon is due to a greater resistance to unfavorable conditions and, above all, to a lesser influence of the annual climatic fluctuations (Maucieri et al., 2018). Further confirmation of this strong stability was observed in the 2019-20 growing season, as even without phytosanitary treatments and fertilization due to the COVID 19 pandemic, quite good reproductive growth parameters were observed in the traditional varieties, while the Claudio variety did not grow due to strong competition from weeds.

A major characteristic that makes traditional varieties suitable for climatic changes is their strong resistance to pathogens. Indeed, as can be observed, they have a lower seed infection rate than the modern Claudio varieties, which is significantly lower. In this case, a significant variability was observed within the group of traditional varieties. In fact, the Senatore Cappelli variety has a higher infection rate than the other traditional varieties, especially Einkorn, Verna and Timilia. According to Yao et al. (2008), the presence of the genes Mlm2033 and Mlm80, which were removed by genetic improvement, is the main cause of the pronounced resistance of Einkorn, Timilia and Verna.

In order to mitigate climate change, these varieties can be used not only in purity, but also to create highly productive varieties that are naturally resistant to ever-increasing abiotic stress factors and, above all, to the pressure of weeds and pathogens that are becoming increasingly aggressive.

a particular method of genetic improvement, evolutionary genetic improvement, in which a mixture of selected seeds is sown in the field instead of pure varieties (Ceccarelli, 2016). Thanks to the great genetic diversity and spontaneous hybridization between varieties, the seed produced is not genetically identical to the seed sown. The seed obtained from this mixture is called an evolutionary population (Ceccarelli, 2016). In contrast, in conventional genetic improvement, new cereal varieties are selected by researchers in experimental stations in a process that takes 10 years, and only at the end of this time are the final products used in farmers' fields (Ceccarelli, 2016).

The main strength of Evolutionary Genetic Improvement has 3 advantages:

a) Complementarity, genetically heterogeneous populations use the limited resource in a different way by reducing competition. For example, use genotypes with different root structures or with different light requirements (Finckh et al., 1997).

b) Cooperation, strains that are tolerant to certain stress factors and pathogens can induce resistance in the most susceptible varieties. For example, some genotypes of *Hordeum vulgare* L. are able to induce resistance to *Rhopalosihum padi* L. virus in susceptible cultivars (Yachi and Loreau, 1999). When susceptible genotypes are separated from each other, there is also less spread of the pathogens among the plants (Finckh et al., 1997).

c) Compensation, sowing in a mixture allows harvesting even if a particular genotype fails. Genetic diversity thus offers a guarantee in an environment constantly plagued by climate change (Yachi and Loreau, 1999). The use and marketing of food based on traditional varieties can be an important economic resource, as the marketing of ancient grains is steadily increasing; since 2018, the market for traditional varieties has increased by 250%, representing a value of approximately €1.667 billion. This increase is due to the growing awareness of customers to consume only high-quality products that have a better nutritional profile compared to the modern wheat varieties currently on the market (Bencze et al., 2020).

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Evaluation of the sustainability of cultivation of Traditional Varieties based on emergy analysis

Abstract

Food security is one of the fundamental issues on which global politics is focused. In 2015, the 2030 Agenda for Sustainable Development was established, with Goal Number 2 being to provide sufficient and quality food for all. However, modern agriculture, which was considered the paradigm for achieving food security, is no longer sustainable due to the excessive use of external inputs and, above all, extreme vulnerability to climate change. Recent studies have shown that agrobiodiversity needs to be promoted in order to mitigate and adapt agriculture to climate change. To this end, traditional cereal varieties are being reintroduced, characterized by lower productivity but greater resilience to abiotic and biotic stresses and, above all, a reduced need for agricultural practices and fertilizers. Despite the remarkable properties of traditional varieties, however, it is not clear whether the extensive cultivation of traditional varieties is ecologically and economically sustainable. To this end, this part of the project aims at assessing the ecological and economic sustainability of organic cultivation of traditional varieties using emergy accounting.

Introduction

Food security is expected to become an increasingly important issue in the coming decades as the world population continues to grow and is estimated to reach 10 billion people by 2050 (Godfray et al., 2010; Bommarco et al., 2013). From the 1970s onwards, the green revolution, i.e., a major transformation and industrialization of the entire agricultural production system, which mainly took place in the more developed Western countries, tried to respond to the growing food demand.

In particular, the green revolution introduced a massive use of external inputs, pesticides and pest control agents, the use of agricultural machinery and new technical means. However, the most important innovation was the creation and full dissemination of highly productive and genetically and phenotypically homogeneous crop varieties to facilitate mechanized cultivation (Evenson and Gollin, 2003). Thanks to all these innovations, the green revolution led to a sharp increase in production. For example, rice production in India increased from 60 million tons in 1970 to 135 tons in 2000. In Asia, malnutrition even decreased significantly, from 47% in the 1960s to 17% in the 2000s (FAO, 2021). However, modern agriculture nowadays is no longer sustainable across generations due to an overexploitation of external inputs, in particular water and chemicals, and above all due to the massive emission of pollutants and GHGs (Altieri, 2009). Furthermore, the agricultural sector is one of the most vulnerable to climate change underway, and Gammans et al. (2017) estimate that climate change will cause a decrease of approximately 33% in conventional cereal production by 2050. The main reason for this strong vulnerability of the modern agricultural sector to climate change is its extreme homogenization and industrialization in all areas of production (Altieri, 2009). In particular, genetically and phenotypically homogeneous varieties are extremely vulnerable to all climate changes and all resulting stress factors. Moreover, the genetic creation of varieties that are not susceptible to herbicides will accelerate the development of increasingly noxious and pesticide-resistant superweeds (Altieri, 2009). This is precisely why recent studies indicate that the most important way to mitigate and adapt to climate change is to promote and enhance agrobiodiversity (Kahane et al., 2013). To this end, the reintroduction of traditional cereal varieties characterized by high genetic diversity, strong resistance to pathogens and lower demand for nutrients and agricultural practices is being explored (Maroni and Ponzini, 2018). Despite these characteristics, the use of ancient varieties has been decreasing, especially because of their lower productivity as compared to modern, high yielding varieties (Moudry et al., 2012; Casañas et al., 2017). Traditional varieties were created through generational selection, i.e., the systematic annual selection of the best plants, best suited to the soil and local climate (Ceccarelli, 2016). Traditional varieties are on average taller and more heterogeneous compared to modern cereals, which make them less suitable for the massive mechanization of agricultural practices. However, they are more competitive against weeds thereby requiring less or no use of environmentally harmful herbicides and fertilizers (Maroni and Ponzini, 2018).

Despite the remarkable properties of traditional varieties, however, it is not clear whether the extensive cultivation of traditional varieties is ecologically and economically sustainable. To this end, this part of the project aims at assessing the ecological and economic sustainability of organic cultivation of traditional varieties and modern varieties using emergy accounting. First introduced by Odum (1988; 1996), the emergy concept makes it possible to quantify all the resource investments required to realize a product - in this particular case the traditional varieties cultivation - in one and the same unit.

Emergy is defined as "the available energy of a kind previously used directly or indirectly to produce a service or product"(Odum, 1988). Through it, the performance of a given system can be assessed based on a common energy metric: in most cases, the solar emjoule (sej), which is developed on the basis of solar equivalent exergy and whose unit is the solar equivalent joule (seJ) (Odum, 1988).

The sum of the emergy demand (energy, matter, labour, information, services) per unit of output is defined as the Unit Emergy value (UEV) and measured in sej/unit (sej/J, sej/kg, sej/h, sej/bit, sej/currency) (Costanza, 1980).

Method

Emergy accounting

Emergy represents all the work done by the environment for a given system to produce a given level of output. The emergy analysis consists of drawing a diagram describing the system with all its inputs, outputs and internal components. Then all inputs and their quantities in joules or grammes are listed in a table. The emergy contribution of each input is then calculated by multiplying the inputs by their (previously calculated) transformations. The total emergy is therefore the sum of all the contributions of the independent inputs (Odum, 1988).

In order to calculate the sustainability of cultivation of traditional varieties through emergetic analysis, the following parameters can be calculated (Cristiano, 2021):

- Emergy yield ratio (EYR), is the ratio between the total emergy yield output (U) and the emergy invested from main economy;
- Emergy investment ratio (EIR), is the ratio between the imported emergy (F) and the local renewable and nonrenewable sources;
- Environmental loading ratio (ELR), compares the orders of magnitude of the emergy requirements from the outer economic systems and from local nonrenewable sources with that of local renewables;
- Emergy sustainability index (ESI), the ratio between EYR and ELR, measures economic and environmental performances altogether;

- Percentage of renewable emergy (% R),

System analyzed

The analysed system of cultivation of traditional cereals varieties is the farm La Battistei, located in Cittadella Municipality (45.674 N, 11.737 E and 70 m elevation), in Northern Italy. The average annual rainfall from 1951 to 2014 at the station was 1429 mm, the average seasonal air temperature was 13.0 °C, and the average sunshine duration was 2978.72 h.

The soil was ploughed to a depth of 20 cm with a tractor-drawn disc plough and large clods of soil were smoothed with a harrow to create a level seedbed.

In both years the following traditional varieties were sown: - "Emmer" *Triticum dicoccum* L., "Einkorn" *Triticum monococcum* L., *Triticum durum* Desf. var Senatore Cappelli, *Triticum aestivum* L. var. Verna and the modern varieties *Triticum durum* Desf. var Claudio . The sowings were carried out on 25 October 2018 and 11-12 November 2020. The identified varieties were sown with double replication in plots of 5 m^2. 250 and 400 seeds per mm2 were sown for the traditional and modern varieties, respectively. During the growing season, 60 g of Super Robur (15%) for Claudio and 30 g for the traditional varieties were applied to the organically grown varieties, particularly on 18 February 2019, 11 March 2020 and 23 April 2020 to support growth. Nitrogen fertilizer, however, was not administered to both einkorn and emmer, as it is not necessary for cultivation, but on the contrary increases the tendency to lodging. To prevent fungal infections, the varieties were sprayed with organic fungicide on 18 March 2019 and 11 March 2021. Finally, mechanical weeding was carried out to eliminate the weeds The harvest took place on 1 July 2019 and 23 June 2021.

Results

The system diagram for our case study is shown in Figure 1. The flows are represented in terms of energy, materials, goods, labor and services. The timeframe is two years. The main process is agriculture, it contributes to the local soil, expressed as stock associated with the process. Agriculture also benefits from renewable sources, although it does not necessarily take full advantage of them. Tools, infrastructures, and structures all fall under the "Agricoltural assets" source of the diagram, i.e., they are imported into the system from external economies, together with vehicles and agricultural machinery (here calculated as annual depreciation based on their useful life), which in its time requires another input: Fuel (here diesel). As in the practice of the system diagram, all imported inputs involve services, i.e., indirect labor previously required (outside the system) to produce the products, their delivery and / or for the associated facilities and infrastructures.

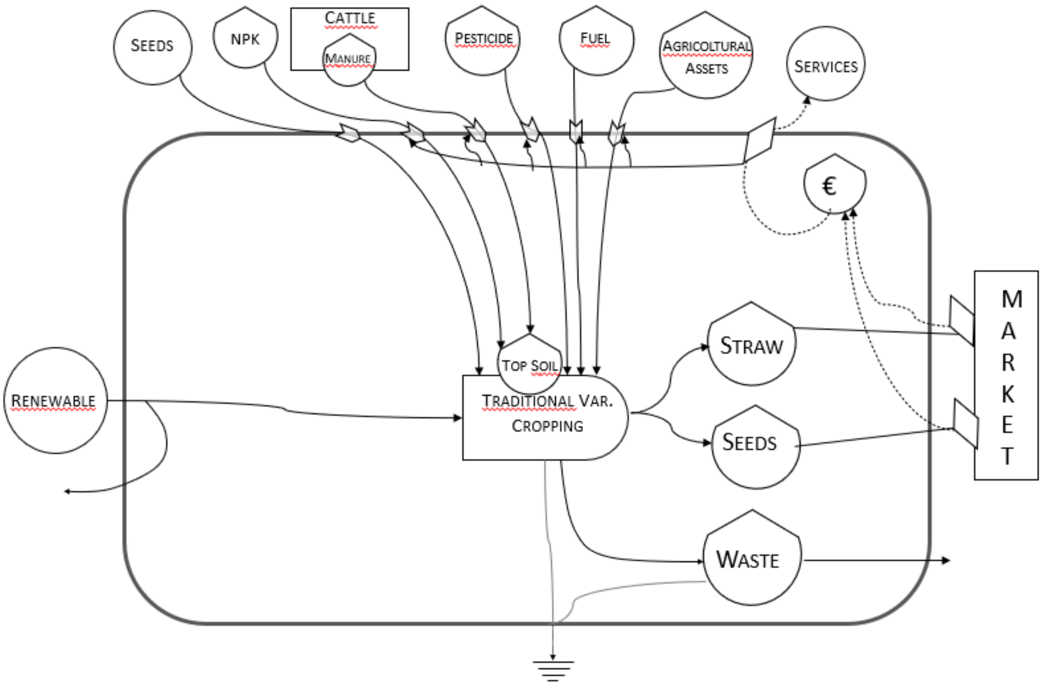

Figure 6 System Diagram

The first quantitative part of emergy accounting starts with the inventory and its emergy transformation, as shown in Table 1. The flows are divided into: local renewable inputs (R), local non-renewable sources (N), purchased material inputs from the external economy (F) and labour and services (L&S). Where necessary, i.e. for stocks and flows that have a longer lifetime, the raw data refer to one year only. The unit of measure of emergy (UEVs) is sej/yr, values are selected accordingly from the solid existing literature.

Table 1 Emergy table of traditional varieties and modern varities cropping

Note	Item	Raw amount	Unit	Timeframe(yrs)	Lifetime (yrs)	Annual amount (unit/yr)	UEV (seJ/unit) [*]	Ref.	Solar Emergy (sej/yr)
CROPPING INPUTS FOR ANCIENT GRAIN PRODUCTION									
Local renewable sources - (R)									
Primary renewable sources									
1	Solar radiation		J	3		2.29E+13	1.00E+00	Brown and Ulgiati (2016)	2.29E+13
2	Deep Heat		J	3		5.67E+09	4.90E+00	Brown and Ulgiati (2016)	2.78E+10
Secondary renewable sources									
3	Wind (kinetic energy)		J	3		1.77E+09	7.90E+02	Brown and Ulgiati (2016)	1.40E+12
4	Rain (chemical potential)		J	3		4.84E+08	1.28E+04	Brown and Ulgiati (2016)	6.20E+12
Total renewable input (largest of renewables)						2.25E+09			
Local nonrenewable sources (N)									
5	Top soil organic matter		J	3		3.66E+07	9.37E+04	De Vilbiss and Brown (2015)	3.43E+12
Imported inputs (F)									
Agricultural assets (structures, infrastructures, tools, and machinery)									
	Shed	200000	g	3	20	6.67E+04	1.10E+01	Buranakarn (1998)	7.33E+05
	Machinery	1.18E+07	g	3	10	1.18E+06	3.38E+09	Cabezas et al. (2010)	3.97E+15
	Tools	10000	g	3	10	3.33E+03	2.40E+09	Buranakarn (1998)	8.00E+12
Grain production									
	Seeds		g		1	9.40E+02	2.40E+12	Fahd et al. (2012)	2.26E+15
	Straw		g		1	2.25E+04	1.71E+15	Zhang and Ma (2015)	3.85E+19
	Manure		g		1	1.25E+05	6.41E+12	Spagnolo et al. (2020)	8.01E+17
	Standard organic nutrients (NPK)		g		1	1.50E+03	6.41E+12	Spagnolo et al. (2020)	9.62E+15
	Pesticide, natural		g		1	2.50E+03	1.60E+09	Xi and Qin(2009)	4.00E+12
Transport inputs									
	Diesel for farming machines		g		#	1.57E+00	1.90E+12	Brown and Ulgiati (2016)	2.98E+12
ASSOCIATED SERVICES (adjusted to one year)									
	Services		€		3.09	2.50E+02	2.40E+12	National Environmental Accounting Database for Italy in the year 2014	6.00E+14
								Subtotal annual emergy, w/out labour & services (U_a)	3.93E+19
								Subtotal annual emergy, with labour & services (ULS_a)	3.93E+19

All emergy values are given both with and without labour and services, i.e., 3.93 E19 sej/kg without labour and services and 3.93 E 19 sej/kg with labour and services.

The specific emergy is instead particularly suitable for comparisons with the same crops grown elsewhere and/or with other food products with similar specific energy content. Finally, some emergy sustainability indicators are calculated and presented in Table 2 for Senatore Cappelli and Verna in the first column and for Einkorn and Emmer in the second column, since both Emmer and Einkorn were not fertilized, Claudio in the third column.

Table 2 Emergy sustainability indicators.

	Verna - Senatore Cappelli	Einkorn - Emmer	Claudio
EYR	1.00E+00	1.00E+00	1.00E+00
EIR	2.20E+04	5.17E+02	4.34E+04
%R	3.72E-05	1.58E-03	1.88E-05
ELR	2.69E+04	6.34E+02	5.31E+04
ESI	3.72E-04	1.58E-03	1.88E-05

Discussion

In particular, from the analysis of the emergy sustainability of emergy production, it appears that in all three cases analyzed EYR, i.e. the Emergy Yield Ratio is close to 1, indicating that the local resources provided are relatively low compared to the inputs still imported (Cristiano, 2021), suggesting that the local resources available are not sufficient for the whole process, since cultivation is an anthropogenic activity in each case. The analysis of the Emergy Investment Ratio indicates the ability and the efficiency to use the emergy present in the system. Table 2 shows that Einkorn and Emmer have a higher EIR than Verna, Senatore Cappelli and Claudio, indicating a greater ability to harness the energy of the system. The Environmental Loading Ratio (ELR) indicates the use of environmental services by a system and reflects the burden the system places on the environment. EYR Values above 10 indicate that the existing ecosystem is under high stress, but even in this case, ELR values indicate that cultivation is generally not a spontaneous process and causes major changes in the natural ecosystem (Cristiano, 2021). It should be noted, however, that the stress of cultivation on the external environment is lower for Einkorn and Emmer, followed by Verna and Senatore Cappelli and Claudio. When evaluating the percentage of renewable emergy used (% R), we observe the same trend as before, i.e. greater use, i.e. greater sustainability for Einkorn and Emmer, followed by Verna, Senatore Cappelli and Claudio. The Emergy Sustainability Index, i.e. the ratio between EYR and ELR, indicates the long-term sustainability of the process under consideration. Values of ESI below 1, as in this case, indicate long-term unsustainability (Crisitiano, 2021), however, it can be observed that Einkorn and Emmer are more sustainable than the other 2 processes studied. This part of the project shows that the cultivation of traditional varieties is not sustainable in the long term. Despite the lower environmental impact, cultivation is based on external inputs managed by the farm. However, it is found that Einkorn and Emmer are more sustainable than Verna, Senatore Cappelli and especially the modern variety Claudio.

Traditional varieties adapt to an agro-ecological approach, i.e. an approach that aims to minimise external inputs, promote inter-trophic interactions and support ecosystem services (Altieri, 2009). For this purpose, it is interesting to use Emergy Accounting to evaluate the sustainability of traditional varieties in the agroecological approach.

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Conclusion

The various laboratory tests have shown that traditional varieties are more dynamic and evolvable than genetically homogeneous varieties due to their high genetic diversity, crucial traits that could improve the resilience and resistance of agroecosystems (Jarvis et al., 2016; Vetelainen et al 2009; Nevo and Chen, 2010). Our research has shown that the traditional varieties have high seed viability and high germination capacity even under different stress conditions, while the modern variety Claudio has always shown a lower germination capacity. Based on these results, we can conclude that these varieties are characterized by greater stress tolerance than the modern varieties. Although the tested traditional varieties showed a high and stable germination rate under increasing stress, they were characterized by different germination reactions. In particular, Verna, Einkorn, Turanico and Timilia proved to be more stress resistant than Senatore Cappelli and Emmer. This characteristic can be explained at the physiological level by a greater production capacity of the metabolite ABA (abscisic acid), which favors the response to stress and water uptake at the beginning of the germination process, so that the percentage of germination remains stable even at high stress levels (Maucieri et al., 2018).

The stress tolerance heterogeneity of the tested cultivars could be a consequence of anthropogenic homogenization of crops and genetic erosion (Jarvis et al., 2016, Heinemann et al. 2014). Indeed, Senatore Cappelli and Claudio, the two least stress-tolerant varieties, have a common origin, since Claudio, like most modern durum wheat varieties, resulted from the mutation of Senator Cappelli by irradiation with gamma and neutron rays (Amicabile, 2016). In contrast, the other varieties (De Cillis, 1927; Maucieri 2018) did not undergo breeding processes and have undergone less intense genetic improvement.

In all the varieties tested, the applied stress delayed and slowed down germination, but with less intensity in Verna, Einkorn, Timilia, Turanico, Emmer and Gentil Rosso than in Senatore Cappelli and Claudio. Slow and delayed germination could lead to pronounced heterogeneity in plant height, resulting in more difficult mechanization of operations such as harvesting, herbicide, pesticide and fertilizer application (Amicabile, 2016).

A severe reduction in seed germination caused by climate change will result in current planting densities being insufficient to maintain current productivity levels (Wang et al., 2015); seed densities would need to be increased from 25% to 150% to maintain the same level as modern cereal production, resulting in an unsustainable economic loss.

The experiment of growing seedlings under stress conditions confirmed that salt critically affects the growth and accumulation of biomass in the post-emergence phenological stages, as highlighted in previous studies (Almansouri et al., 2001). This can be a critical problem for conventional forage producers who need high biomass plants to support livestock production. However, the results also showed that the traditional varieties lost less height and biomass under stress conditions, both in absolute and percentage terms, than the modern variety Claudio. This strong salt tolerance could be due to the improved ability to regulate osmotic potential and control cellular levels of ROS (Miransari and Smith, 2019).

Based on the results collected, the varieties analyzed can be classified into 3 use categories: **Recovery and upgrading of degraded areas** (Verna, Einkorn, Timilia, Turanico), moderately **stressed areas** (Emmer, Gentil Rosso), **non-stressed areas** (Senatore Cappelli, Claudio).

The part of the project that looked at the interaction of nitrogen and yeasts as a means of mitigating salt stress showed that certain yeast strains and the use of nitrogen compounds can reduce the effects of salt on germination, more so in the modern variety Claudio than in the traditional varieties. Despite the slight attenuating effect observed in the traditional varieties, they had a higher germination capacity than the modern variety Claudio.

Field trials confirmed the lower productivity of traditional varieties compared to modern varieties, due to several factors:

- Lower sowing density tolerated by traditional varieties
- Lower hectoliter weight of seed
- Lower number of seeds per year
- Greater susceptibility to pathogens

However, traditional cultivars have shown exceptional resistance to pathogens and vigorous vegetative growth with high green biomass content even at advanced phenological stages. The Ca' Foscari University chemistry group evaluated the traditional varieties from a nutritional point of view and found a better nutritional profile (data not published). In fact, the content of amino acids, flavonoids and trace elements is higher in the traditional varieties than in the modern ones. Both human and animal diets based on the use of traditional varieties can result in significant benefits:

- More digestible and agreeable foods, as they are characterized by a lower gluten index (Ghiselli et al., 2016).
- Nutrition more suitable for diabetics because they do not increase the glycemic index and reduce cholesterol (Ghiselli et al., 2016)
- Reduction in the risk of anemia and skeletal fragility in cattle and increased milk production, fetal development and survival (Amicabile, 2016).

Jarvis et al. (2016) stated that traditional varieties will be crucial for future food security, as their reintroduction will enable an exponential increase in biodiversity at both within and between agroecosystems. Indeed, ecosystems with high agrobiodiversity are characterized by greater resilience and resistance to climate change, as the cultivation of traditional varieties allows for constant production over time, even when exposed to increasing stress (Kahane et al., 2013). This characteristic is particularly important for developing countries, which are increasingly affected by climate change and which, above all, do not have the high resources and inputs to cope with and mitigate such phenomena. Moreover, their use has another advantage, because seeds of traditional varieties are independent of the current oligopoly of multinational seed companies. Indeed, these large companies determine the management of seeds, ignoring the needs of producers and consumers and focusing only on profit (Aerni, 2011). As a result, small farmers without large resources are forced to grow only a few varieties or use inferior seeds, leading to health problems (Altieri, 2009). The use and marketing of food based on traditional varieties can also be an important economic resource in the Mediterranean region, as the marketing of ancient cereals is steadily increasing; since 2018, the market for traditional varieties has increased by 250%, representing a value of approximately €1.667 billion. This increase is due to the growing awareness of customers to consume only high-quality products that have a better nutritional profile compared to the modern wheat varieties currently on the market. Further studies need to be conducted to test and discover other varieties to utilize and preserve this ancient genetic heritage. By understanding how climate change affects crop production and how different varieties respond to abiotic stresses, we can ensure crop productivity while avoiding degradation of arable land and conserving natural resources.

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