**RESEARCH ARTICLE** 



# Multidisciplinary study of a little known landrace of *Fagopyrum tataricum* Gaertn. of Valtellina (Italian Alps)

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**Abstract** A little known landrace of *Fagopyrum tataricum* Gaertn. ("Grano Siberiano Valtellinese") introduced at the end of the eighteenth century in the mountain areas of Valtellina (Northern Italy) has been analysed according to agronomical (plant height, number of flowers per plant, germinability and weight of the seeds), ecological (plant functional strategy), phytochemical (rutin, quercetin and fagopyrin content) and historical characteristics and compared it with other genotypes of *F. tataricum* and *F. esculentum* Moench. Analysis showed that "Grano Siberiano Valtellinese" is the genotype best adapted to the environmental conditions of the Valtellina and the

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Department of Agricultural and Environmental Sciences -Production, Landscape and Agroenergy, University of Milan, Via Celoria 2, 20133 Milan, Italy most tolerant to stress (functional strategy: CS/CSR). It has a higher concentration of rutin in the seeds  $(764 \pm 39 \ \mu g/g)$  and in the shoots  $(370 \pm 66 \ \mu g/g)$  which make it interesting for the production of nutraceutical foods. In order to protect this landrace, its inclusion in the European Register of Conservation Varieties has been proposed.

Keywords Buckwheats · Rutin · Quercetin ·

 $Fagopyrin \cdot Landraces \cdot Nutraceutical \ food \cdot Mountain \ agriculture$ 

### Introduction

The conservation of agrobiodiversity is a subject of considerable interest which nowadays involves various parties (consumers, farmers, researchers, associations, public bodies and institutions) from a local to a global scale. The safeguarding of agrobiodiversity is an extension of the concept of biodiversity conservation that refers specifically to the varieties/races of plant and animal species of agricultural interest. Unfortunately, many of these genetic resources (both animal and plant) have been lost over time, either because replaced with other more productive varieties/ races or due to changing consumer tastes. As regards plant agrobiodiversity, which today represents one of the richest and least explored sectors of agricultural diversity, the FAO estimates that in the last century, more than 75% of the genetic diversity of agricultural crops worldwide has been lost (FAO 2004). In fact, in the past there were many more landraces (understood as dynamic populations of cultivated plants that have historical origin and distinct identity, and lack formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems (Camacho Villa et al. 2005) that, besides characterizing the agri-food and historicalcultural aspects of the various areas in which they were cultivated, constituted unique genetic resources. Today, given the interest of consumers in quality local agri-food products, there is, at least in some areas of the world, a re-evaluation of some landraces that have been preserved in situ (by farmers) and/or ex situ (in seed banks). This re-evaluation is reflected in an increase in their areas of cultivation due to the creation of small chains of unique traditional and innovative products supported by national and international conservation and development programs and initiatives. The rediscovery and re-evaluation of almost totally abandoned varieties has also stimulated the attention of the scientific community as many landraces are today known little or not at all (as regards the various genetic, agronomic, phytochemical, ecological, historical aspects, etc.) and could be of great interest as material to be used in genetic improvement plans of crops. One example is the "Grano Siberiano Valtellinese", a little known annual self-pollinated landrace of Tartary buckwheat (Fagopyrum tataricum Gaertn.), introduced since the late eighteenth century (Giacomini 1954) in the mountain areas of Valtellina (Lombardy, Northern Italy) and now at risk of extinction since it is cultivated by an extremely limited number of farmers (less than 10) working mostly for a hobby. The reasons that led to the abandonment of this crop, as well as many others, are various but are mainly due to the abandonment of mountain areas by human beings. This phenomenon has intensified in the Alps since the middle of the last century and it is still in progress (Laghetti et al. 1993; NORDREGIO 2004; Keenleyside and Tucker 2010; Terres et al. 2013). The abandonment of mountain areas (and the connected agricultural practices) has caused changes to the landscape (loss of agricultural land due to the expansion of forests), imbalances to ecosystems (increase in susceptibility to hydrogeological instability phenomena), and the loss of agrobiodiversity with everything connected with it (typical dishes, traditions and popular culture linked to agriculture). In recent decades, there was also a desire to favor the exclusive cultivation of the common buckwheat (*Fagopyrum esculentum* Moench) in Valtellina and hence discourage the cultivation of "Grano Siberiano Valtellinese", since the former is more appreciated by consumers for the preparation of "pizzoccheri"—a traditional dish of Valtellina today mainly produced with common buckwheat flour of Polish origin or, more rarely and mainly for family use, using flour from the two local landraces of *F. esculentum* ("nustran" and "curunin") (Barcaccia et al. 2016).

The most probable birthplace of cultivated Tartary buckwheat is the Yunnan province of China (Tsuji and Ohnishi 2000) where it is traditionally cultivated also at high altitude (on the Loess and Yungui plateaus in the western part of the country) on thin soils poor in nutrients (Lin et al. 2006). In Asia (especially in China and neighboring countries) and in other parts of the world where it is common, it is grown for the production of healthy and functional food and as a medicinal plant due to its well-known health-promoting and nutraceutical properties (Kreft et al. 2003; Wang and Campbell 2007; De Rossi et al. 2013; Ikeda et al. 2017). These properties are due to the polyphenol content, especially rutin, which is very high in F. tataricum compared to F. esculentum (Fabjan et al. 2003; Brunori and Végvári 2007; Jiang et al. 2007; Kim et al. 2008; Gupta et al. 2012; Zielinska et al. 2012; Tolaini et al. 2016). Rutin is used to prevent and cure vein diseases, serum cholesterol, retinopathy and fatigue symptoms (Archimowicz-Cyryłowska et al. 1996; Ihme et al. 1996; Wieslander et al. 2011; Ikeda et al. 2017). Apart from rutin, buckwheat also contains fagopyrin and quercetin. While the former is a molecule which causes sensitivity to light after ingestion of large amounts of green parts (leaves, stems and flowers) of buckwheat (Hinneburg and Neubert 2005; Stojilkovski et al. 2013), the latter is produced thanks to an enzyme which degrades rutin (flavonol 3-glucosidase), together with other compounds, gives Tartary buckwheat flour its bitter flavour (Suzuki et al. 2004).

Compared to eastern countries, in Europe and in the Alps in particular, Tartary buckwheat is not widespread nowadays (Hammer et al. 1999; Brunori et al. 2006) and is considered more a common buckwheat weed than a crop in itself. This research therefore aimed to:

- Analyze the agronomic characteristics of "Grano Siberiano Valtellinese", comparing them with those of other varieties of *F. tataricum* (from other geographical areas) and the two landraces of *F. esculentum* of Valtellina (Barcaccia et al. 2016) grown in experimental fields (in Valtellina), in order to highlight the characteristics of this landraces.
- Evaluate the CSR (C—competitors, S—stress tolerator, R—ruderals) functional strategy of "Grano Siberiano Valtellinese" (and the other plant materials considered in this paper), according to the theory of Grime (1974, 1977, 2001), in order to define the ecological adaptations adopted by this landrace in response to the environmental characteristics of the area where it was cultivated in recent centuries.
- Analyze and compare the content of rutin present in the seeds and shoots of "Grano Siberiano Valtellinese" and other genotypes of *F. tataricum* and *F. esculentum* in order to evaluate the nutraceutical properties of the fruits and shoots that are the main parts of the plant used in human nutrition (Kim et al. 2004).
- Collect historical information (both bibliographic and direct testimonies) on the introduction, selection and cultivation of "Grano Siberiano Valtellinese" in Valtellina (and surrounding areas) as this information is necessary to register this landrace in the European Register of Conservation Varieties (Spataro and Negri 2013) which is one of the main European tools for safeguarding plant agrobiodiversity.

This research was carried out in compliance with the objectives of an agreement between CRC Ge.S.Di.-Mont. Research Center and Lombardy Region aimed at characterizing and promoting the little known landraces present in the mountain territories of Lombardy, therefore it represents the joint work between a research institute, a territorial management body and the farmers who have cultivated (and preserved) "Grano Siberiano Valtellinese".

## Materials and methods

### Experimental fields and plant material

In order to compare the agronomic, ecological and phytochemical characteristics of "Grano Siberiano Valtellinese" with other genotypes of F. tataricum and F. esculentum, 6 experimental fields were set up in 2017 in Teglio (Sondrio, Valtellina, latitude: 46°10'25"N, longitude: 10°02'48"E) located at an altitude of about 900 m a.s.l. and at a distance of more than 200 m from each other in order to avoid crosspollination. Conditions (inclination, exposure, soil) were maintained as far as possible similar in each field and one of the six genotypes listed in Table 1 was cultivated in each. Figure 1 shows the experimental field of "Grano Siberiano Valtellinese". In the 25 m<sup>2</sup> plots, 300 g of seeds were sown by broadcasting in the open at the beginning of August 2017 (after the rye harvest). The sowing period and the amount of seeds (120 kg of seeds per hectare) was assessed on the basis of the testimonies of local farmers, thus following traditional techniques. The fields were fertilized in October 2016 using 1 kg/m<sup>2</sup> of manure and were not irrigated. Table 2 shows the average monthly rainfall and temperatures of Teglio for the year 2017. The territory where the experimental fields were set up belongs to the temperate continental bioclimate (Rivas-Martínez 2004).

Agronomical analysis and functional strategy evaluation

In each experimental field, data and plant samples were collected in order to compare the agronomic and biological characteristics of the six genotypes. The following agronomic characteristics were evaluated:

- Plant height—the shortest distance between the upper boundary of the main photosynthetic tissues (including apical inflorescences) on a plant and the ground level was measured considering 50 plants chosen randomly in each experimental field. Plant height was measured during the period of full flowering (mid-September 2017 for *F. tataricum* and the end of August for *F. esculentum*) using a measuring rod.
- Number of flowers per plant—for each of the 50 plants whose plant height was measured, the

Code	Species	Name/code of genotype	Source of seeds	City of seed origin	Country
A	Fagopyrum tataricum	Grano Siberiano Valtellinese	"Raetia Biodiversità Alpine" Farm	Teglio (Sondrio)	Italy
В	Fagopyrum tataricum	-	Bozhou Swanf Natural Product Co.	Bozhou (Anhui)	China
С	Fagopyrum tataricum	LFE003 <sup>a</sup>	Laimburg Research Center	Vahrn (Bolzano)	Italy
D	Fagopyrum tataricum	LIFAGO	Die Deutsche Saatveredelung AG (DSV)	Lippstadt	Germany
Е	Fagopyrum esculentum	Nustran	"Raetia Biodiversità Alpine" Farm	Teglio (Sondrio)	Italy
F	Fagopyrum esculentum	Curunin	"Raetia Biodiversità Alpine" Farm	Teglio (Sondrio)	Italy

 Table 1
 Data of plant materials

<sup>a</sup>Code of the seed bank of Laimburg Research Center



Fig. 1 "Grano Siberiano Valtellinese" landrace: plants in the experimental field (a) and seeds (b)

flowers were counted so as to assess the relationship between plant height and number of flower of each genotypes.

**Table 2** Temperature (T), precipitation (P) and potential evapotranspiration (PE) from S. Giacomo (Teglio) weather station (2017) *Source*: ARPA Lombardia. PE was calculated using the online diagnosis tool developed by Rivas-Martínez and Rivas-Sáenz (2009)

	T (°C)	P (mm)	PE (mm)	P – PE (mm)
January	- 3.6	4.2	0.0	4.2
February	4.0	57.6	9.8	47.8
March	9.9	33	39.5	- 6.5
April	12.4	62.8	58.2	4.6
Maj	16.5	129.6	95.6	34.0
June	21.1	223.2	131.6	91.6
July	21.3	61	135.3	- 74.3
August	21.1	123.6	123.5	0.1
September	14.7	120	66.5	53.5
October	11.0	3.4	41.6	- 38.2
November	4.1	54.6	10.0	44.6
December	- 1.9	140.2	0.0	140.2
Year	10.9	1013.2	711.6	301.6

- 1000-seed weight—assessed by weighing (using analytical balance Precisa XB 220A) a sample of 50 seeds per genotype. The test was performed in triplicate.
- Germinability—number of germinated seeds compared with total number of seeds placed on Petri dishes after 7 days. During this period of time the dishes were left at room temperature (20 °C) in natural light and watered regularly with distilled

water. Germination test was performed in triplicate seeding 25 seeds per dishes.

The analysis of the CSR functional strategy (Grime 1974, 1977, 2001) of each genotype was performed according to the latest method proposed by Pierce et al. (2017). In detail, in each experimental field 15 fully expanded leaves, taken from different plants, were collected in September 2017. These leaf samples were wrapped in moist tissue paper and stored in the dark overnight at 4 °C. Leaf fresh weight (LFW) was determined from these saturated organs using analytical balance (Precisa XB 220A) and the leaf area (LA) was measured using digital scanner and the ImageJ 1.52a software. Leaf dry weight (LDW) was measured after oven (MPM Instruments M40-VN) drying at 105 °C for 24 h. CSR coordinates and CSR strategy were determined using 'StrateFy' tool (Pierce et al. 2017) and were projected in the CSR ternary graph using the 'ggplot2' package of R version 3.5.1 (R Development Core Team 2018).

## Phytochemical analysis

For each of the six genotypes, the content of rutin, quercetin and fagopyrin in the seeds and in the seedling shoots at various stages of growth (4, 9 and 11 days after seeding) was evaluated with high performance liquid chromatography (HPLC) analysis. The shoots of the six genotypes were produced by placing the seeds on Petri dishes to make them germinate. Distilled water was sprayed on the seeds every day. Seedling shoots were collected 4, 9 and 11 days after germination. Flavonoids extraction was performed according to the method of Stojilkovski et al. (2013): a portion of 0.800 g of pulverized sample of seeds or shoots was refluxed with 40 ml of methanol for 4 h at 65 °C. The extractions were performed in triplicate. The extracts of seeds and shoots were filtered with 0.45 µm nylon filters and were analyzed by HPLC. Chromatographic analysis was performed with a liquid Chromatograph Dynamax SD200 (VARIAN<sup>®</sup>), equipped with a binary pump with Rheodyne injector and a UV-Vis detector managed by the Galaxy chemstation. A reversed phase column C18 Hyperil ODS (300 mm length, 4.0 mm ID, 5 µ, Termo Fisher) was used. The HPLC conditions used were as follows: flow 0.8 ml/min,  $\lambda = 353$  nm; water with trifluoroacetic acid (TFA) 1% (A), acetonitrile (B); gradient elution initially 10% of B for 5 min, 60% of B at 60 min, at 45 min 30% of A. Fagopyrin was detected at 590 nm using an elution gradient from 50% of B increased to 100% in 20 min. The fagopyrin content was evaluated using hypericin standards because a fagopyrin standard was not available. A mixture of hypericin and pseudohypericin (2:1 by HPLC) were isolated from commercial St. John's Wort (*Hypericum perforatum* L.) according to Ramezani and Zamani (2017). Solvent and standards of rutin (95%) and quercetin (95%) were purchased from Sigma-aldrich (Italy).

## Statistical analysis

Agronomic, ecological (functional strategy) and phytochemical data referring to each genotype, expressed as the mean  $\pm$  SD (standard deviation), were ranked using Principal Component Analysis (PCA) in order to highlight the main variables that differentiated the samples. PCA was performed using the "vegan" package (Dixon 2003) of R 3.5.1 software (R Development Core Team 2018).

# Historical data collection

Information regarding the introduction, selection, cultivation techniques and uses of "Grano Siberiano Valtellinese" was collected by bibliographic research and interviewing elderly farmers from Valtellina and surrounding areas. In addition, an online questionnaire was created (http://www.unimontagna.it/agrobiodiversita-vegetale-italiana/) to highlight the presence of farmers who still cultivate this landrace. The questionnaire was sent to over 15,000 contacts spread over the territory of the Lombardy Region and nearby areas.

# Results

The results of the agronomic, ecological and phytochemical analyzes concerning "Grano Siberiano Valtellinese" and the other genotypes with which it was compared are reported. Figure 2 shows the graphs of the height and number of flowers of the six studied genotypes. The "Grano Siberiano Valtellinese" (A) has tall plants, 82 cm on average, with around 310 flowers. Genotype D and the two landraces of *F*.



Fig. 2 Number of flowers and height of the plants of the six genotypes considered: **a**, "Grano Siberiano Valtellinese"; **b**, *F. tataricum* of China; **c**, *F. tataricum* LFE003; **d**, *F. tataricum* 

*esculentum* (E and F) show similar characteristics, albeit with slightly lower values. Genotype B, instead, presents tall plants (average height: 95 cm) but with a low number of flowers (average number of flowers: 45) while genotype C presents plants with reduced dimensions (average height: 21 cm) and a low number of flowers (average number of flowers: 39). Table 3 shows the values of 1000-seed weight and germinability of the six genotypes. A has heavier seeds with fairly good germination while B is the one with the lightest seeds and the highest germination.



LIFAGO; **e**, *F*. esculentum "nustran"; **f**, *F*. esculentum "curunin". Trend line and coefficient of determination  $(R^2)$  are shown

The CSR graph and the mean CSR strategy of the six genotypes are reported in Fig. 3 and in Table 4. The CSR values are quite different among the various genotypes of *F. tataricum*, in particular A was found to be the most stress tolerator and the least ruderal while B is the most competitor and C the most ruderal. The two landraces of *F. esculentum* (E and F), on the other hand, have a very similar functional strategy. The B and C genotypes do not show any adaptation to stress, understood as phenomena which restrict photosynthetic production (Grime 2001).

**Table 3** 1000-seed weight and germinability of the six genotypes: A, "Grano Siberiano Valtellinese"; B, *F. tataricum* of China; C, *F. tataricum* LFE003; D, *F. tataricum* LIFAGO; E, *F. esculentum* "nustran"; F, *F. esculentum* "curunin"

Genotype	Average	± SD
1000-seed weight (g	g)	
А	25.37	2.23
В	8.44	4.56
С	19.96	1.68
D	22.55	2.95
Е	22.67	3.99
F	14.59	3.67
Germinability (%)		
А	81.33	20.13
В	97.33	2.31
С	81.33	14.05
D	85.33	14.05
Е	82.67	8.33
F	77.33	4.62



Fig. 3 CSR ternary graph. Key: A, "Grano Siberiano Valtellinese"; B, F. tataricum of China; C, F. tataricum LFE003; D, F. tataricum LIFAGO; E, F. esculentum "nustran"; F, F. esculentum "curunin"

The results of the HPLC analysis of seed and shoot extracts are different for the six genotypes. In particular, the rutin content in the seeds of the 4 genotypes

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Table 4 CSR strategy of the six genotypes: A, "Grano Siberiano Valtellinese"; B, *F. tataricum* of China; C, *F. tataricum* LIFA003; D, *F. tataricum* LIFAGO; E, *F. esculentum* "nustran"; F, *F. esculentum* "curunin"

Genotype	C (%)	S (%)	R (%)	Strategy
А	43.1 ± 7.1	37.4 ± 12.0	19.5 ± 9.1	CS/ CSR
В	$65.0\pm8.8$	$0.0 \pm 0.0$	$35.0\pm8.8$	C/CR
С	$17.3\pm5.3$	$0.0\pm 0.0$	$82.7\pm5.3$	R/CR
D	$36.7\pm8.0$	$32.2\pm8.2$	$31.1\pm9.0$	CSR
Е	$43.6\pm5.4$	11.3 ± 12.0	45.1 ± 10.7	CR/ CSR
F	$44.1\pm8.8$	4.9 ± 11.9	$51.0 \pm 14.8$	CR

of F. tataricum is much greater than that of the F. esculentum landraces (Fig. 4), this confirms data published in the literature (Brunori and Végvári 2007; Jiang et al. 2007; Kim et al. 2008; Gupta et al. 2012; Zielinska et al. 2012). Among the genotypes of F. tataricum, B is the one with the highest content of rutin followed by A (slightly less than B), C and D. The content of rutin is different in the shoots of the genotypes considered (Fig. 5) despite having the same trend according to the stage of development of the shoots. In fact, for all genotypes, with the exception of F, the highest content of rutin is 4 days after sowing, then decreasing in the 9-day shoots to increase again in the 11-day ones. Similarly to the observations for the seeds, the content of rutin is much lower than that of the F. tataricum genotypes also in the shoots of



Fig. 4 Rutin content in the seeds of the four genotypes of *F. tataricum* (grey bars) and in the seeds of the two landraces of *F. esculentum* (white bars). Key: A, "Grano Siberiano Valtellinese"; B, *F. tataricum* of China; C, *F. tataricum* LFE003; D, *F. tataricum* LIFAGO; E, *F. esculentum* "nustran"; F, *F. esculentum* "curunin"



Fig. 5 Rutin content in the shoots of the six genotypes considered at different growth stages (4, 9 and 11 days after sowing). Key: **A**, "Grano Siberiano Valtellinese"; **B**, *F. tataricum* of China; **C**, *F. tataricum* LFE003; **D**, *F. tataricum* LIFAGO; **E**, *F. esculentum* "nustran"; **F**, *F. esculentum* "curunin"

the two landraces of F. esculentum (E and F). Genotype C is the one with the highest rutin content in the 6- and 11-day shoots while the shoots of landrace A are those in which rutin is more abundant on the ninth day. In addition, A is the one that, compared to the other genotypes of F. tataricum, presents a lower variation in the concentration of rutin over shoot development time. In the samples analyzed (seeds and shoots) fagopyrin, a compound found mainly in the green parts (leaves, flowers and stems) of the plant (Stojilkovski et al. 2013) was not detected. Quercetin was found only in low concentrations in landrace E (3.59  $\pm$  0.14 µg/g DW). The low content or absence of quercetin in Tartary buckwheat seeds is in line with findings by Fabjan et al. (2003) and Galvač et al. (2017). Figure 6 shows the chromatogram of the seed extract of sample A compared with the chromatogram of standard rutin and quercetin. The peak of rutin is evident while quercetin is absent as shown in the chromatogram of sample A.

In the PCA biplot (Fig. 7) the main variables which diversify the buckwheat varieties studied can be observed. In particular, the first axis (PC1) distinguishes the *F. tataricum* genotypes from those of *F. esculentum* according to the rutin content in seeds and shoots. The second axis (PC2) is the one that most diversifies the four genotypes of *F. tataricum* for agronomic and ecological characteristics (functional strategy). In fact, A is the one that has taller plants,

with more flowers per plant and is the most stress tolerator while C (placed on the opposite side of the PC2 axis in the graph compared to A) has low stems and ruderal strategy.

From the research of historical information on the introduction and cultivation of "Grano Siberiano Valtellinese" it emerged that this variety was introduced and selected in Bormio (Valtellina, Lombardy) by Ignazio Bardea (1736–1815), a priest and historian of the time, starting from 1786. Bardea received the seeds of this "unknown crop" from a grocer from Brescia in 1735 and, after having cultivated and selected the most productive plants in his vegetable garden, spread it in the upper Valtellina and in the nearby valleys (Valcamonica) with the aim of providing the population with a crop that could be grown in the mountains (even over 1000 m a.s.l.) so as to constitute an integration to the scarce food resources of the time. In fact, this variety could produce up to 1800 kg/ha of grain and 3000 kg/ha of straw in places where it was difficult to grow other agronomic plants. Bardea immediately realized that that cultivar of F. tataricum was much more resistant to the cold compared to the cultivars of F. esculentum that were already cultivated in Valtellina and, in memory of what he had done for the agriculture of the Valtellina mountains, had the following words inscribed on the wall of his vegetable garden: "This vegetable garden was the cradle of the Siberian wheat introduced in Bormio in 1786 by Priest Ignazio Bardea Can.co Teol.o then from Bormio spread in the neighboring provinces and was also transported to France in 1788. By monument Francesco Micheli Brescia Painter 18th June, 1789". The text of this inscription, as well as a lot of other information concerning the introduction, selection and dissemination of this cultivar, is reported in a manuscript of Bardea ("Notions around Siberian buckwheat") kept in the Bormio parish archives. Bibliographic research also revealed an article from the 1950s (Giacomini 1954) reporting historical information related to the cultivation and use of Siberian (Tartary) buckwheat in Valtellina and Valcamonica. It was sown in fields at the highest altitudes (after the harvest of rye or in conjunction with the sowing of potatoes) to produce a flour that was used to feed animals or the poorest peasants. It was also used as fodder grass (mown shortly after the start of flowering). During the last century the cultivation of Siberian buckwheat in Valtellina gradually



Fig. 6 HPLC chromatogram of standard rutin and quercetin (a) and of extract of "Grano Siberiano Valtellinese" seeds (b)

diminished. Already in the 1950s, in fact, few farmers cultivated it in Valtellina and nearby Valcamonica (Giacomini 1954), so it became a "weed" of the *F. esculentum* fields. The testimonies collected by the online questionnaire and from interviews with elderly local farmers have confirmed what was reported by Giacomini (1954), i.e. that the cultivation of "Grano Siberiano Valtellinese" was almost completely

abandoned after World War II because of the overly bitter taste of its flour. Figure 8 shows the map highlighting the area in which, based on the historical information collected, "Grano Siberiano Valtellinese" was cultivated in Lombardy. Currently, farmers and/or hobby farmers who cultivate this landrace are less than ten including Mr. Patrizio Mazzucchelli



**Fig. 7** PCA ordination biplot of samples (capital letters) associated with variables (arrows). Key: A, "Grano Siberiano Valtellinese"; B, *F. tataricum* of China; C, *F. tataricum* LFE003; D, *F. tataricum* LIFAGO; E, *F. esculentum* "nustran"; F, *F. esculentum* "curunin"; height, plant height; flowers, number of flowers per plant; seed\_quer., quercetin content in the seeds; 1000\_s.w., 1000-seed weight; Germ., germinability; seed\_rut., rutin content in the seeds; sprout\_rut.4, rutin content in the shoots 4 days after sowing; sprout\_rut.9, rutin content in the shoots 9 days after sowing; c%, C value; S%, S value; R%, R value. The two broken lines highlight the genotypes of *F. tataricum* and the genotypes of *F. esculentum* 

(custodian farmer) who has cultivated and preserved it for decades in the fields of Teglio.

## Discussion

This research has provided more information on the biology, ecology, chemical compounds and the history of a little known *F. tataricum* landrace: "Grano Siberiano Valtellinese". Analysis has shown that this landrace is the most suitable for the environmental conditions of the mountain areas of Valtellina as, in these areas, it is able to grow higher and produce more flowers than other genotypes of *F. tataricum* coming from other geographical areas. This is undoubtedly due to the selection, both natural and anthropic, made in the last two centuries in which it was cultivated in Valtellina and neighboring valleys. The analysis of its CSR strategy also showed a particular adaptation of this landrace to the territory in which it has been

cultivated. In fact it turned out to be the most stress tolerator among the cultivars considered. This functional adaptation may have been acquired in response to environmental factors that characterize the mountain areas of Valtellina and could be attributable to a family of gene families involved in signal transduction, gene regulation, and membrane transport (Zhang et al. 2017).

In our opinion, the stress to which this landrace would have adapted consists in the low temperatures of the high mountains and the thin soils poor in nutrients and dry. This latter factor could be the one with the greatest impact on the results obtained in this study, in particular on the agronomic findings. The experimental fields, in fact, despite having been set up in a mountain area at mid-altitude (900 m a.s.l.) with fertile soil (fields where cereals and vegetables have been sown for decades), would have suffered from the lack of precipitation during the summer and autumn months of 2017 (as highlighted in Table 2), a typical condition of the continental climate of Valtellina (Isotta et al. 2014). "Grano Siberiano Valtellinese", in addition to being the landrace with the tallest plants and with the greatest number of flowers (and therefore of fruit), has a high content of rutin both in the seeds and in the shoots. This molecule is much more concentrated in "Grano Siberiano Valtellinese" than in the other two landraces of F. esculentum ("nustran" and "curunin") (Barcaccia et al. 2016) of Valtellina which, during the last century, completely replaced the cultivation of "Grano Siberiano Valtellinese" because, as revealed by the historical evidence, "Grano Siberiano Valtellinese" produced a flour that was too bitter. The bitter taste of flour would be due to the presence of quercetin, the bitter compound produced by enzymatic reaction of rutin (Suzuki et al. 2004). Is known that quercetin has specific activity on the bitter taste receptor TAS2R14 (Wiener et al. 2012; Levit et al. 2014) while rutin is inactive towards receptor TAS2R (Mancuso et al. 2015).

On the basis of the results of this research it would be worth valorizing this landrace once again in Valtellina and surrounding areas not so much as an emergency food for the poorest farmers, as it was in the past (Giacomini 1954), but for the high content of rutin and the absence of gluten (which also makes it suitable for celiac diets). The nutraceutical aspect of foods, which in past centuries was not taken into consideration (or indeed not even known), is





nowadays highly appreciated by consumers who are increasingly careful about the quality and health properties of the foods they eat. This is confirmed by the continued expansion of the Italian agri-food sector (and of exports in particular) of which a large part consists of typical high quality products (USDA 2017). Valorising "Grano Siberiano Valtellinese" and the products that derive from it could be a strategic operation that, in addition to contributing to the protection of agro-biodiversity, could lead to the creation of a unique and quality food chain in order to create income for the farmers and restaurateurs of Lombardy mountain territories. This could be a tangible example of the various efforts that the Lombardy Region, the Italian Ministry of Agriculture, Food and Forestry Policies (MIPAAF), and the European Union have made (and are making) to protect agrobiodiversity, enhance local food products, counteract the depopulation of mountain areas and pursue sustainable development (EU Commission 2010). In order to protect "Grano Siberiano Valtellinese" and promote the creation of an agri-food chain it would be worth pursuing the good practices which have been followed (and are being followed) by other Italian mountain landraces (Cassani et al. 2017; Giupponi et al. 2018) summarized in the following three main points:

- Conservation of the landrace by registering it in the European Register of Conservation Varieties (Spataro and Negri 2013) or by using other means of conservation;
- 2. Dissemination of knowledge on the characteristics of the landrace to farmers, restaurateurs and consumers;
- 3. Creation of a quality agri-food supply chain with traditional and innovative products.

The first point is also the first action to be undertaken in order to protect the landrace and the territory in which it was and can be cultivated. In this sense, CRC Ge.S.Di.Mont., in collaboration with custodian farmers and local authorities, presented the application to request the registration of "Grano Siberiano Valtellinese" in the European Register of Conservation Varieties in July 2018. This document, accompanied by a technical scientific report showing the agronomic characteristics of the variety (written according to the guidelines of the International Union for the Protection of New Varieties of Plants (UPOV) and the historical evidence of its cultivation in Valtellina, is now under consideration by the Lombardy Region and the MIPAAF which, in the coming months, will express their opinion on the application submitted. The second point is also essential to preserve this landrace as it is only by spreading information about the characteristics of "Grano Siberiano Valtellinese" that it will be possible to motivate farmers, restaurateurs, and consumers to cultivate, transform and consume it. This will encourage the creation of a supply chain (third point) that, in addition to offering traditional dishes such as Valtellina "polenta" and "pizzoccheri", could propose innovative products such as bread (Vogrincic et al. 2010), pasta, biscuits and functional foods as proposed by ENEA (national agency for new technologies, energy and sustainable economic development) (Tolaini et al. 2016).

"Grano Siberiano Valtellinese" could also be used to make herbal teas or cosmetics (F. tataricum is included in the European Cosmetic Ingredient database (https://ec.europa.eu/growth/sectors/cosmetics/ cosing)) as has been the case for decades in Eastern countries and its seeds could also be sold for the production of edible shoots (Kim et al. 2001, 2004). Shoots could represent an innovative product (at least in Italy and in Europe) since sprouts-based dishes, quite common in Eastern culture, are rapidly spreading even in countries where this culinary tradition is not so widespread. In fact, the sprouts of F. tataricum, as well as being rich in rutin, are abundant in nutrients such as protein, amino acids, and minerals (Ikeda et al. 1995) and could be valorized as functional food (Roche et al. 1999).

# Conclusion

This research has allowed a series of data to be collected on the characteristics of a little-known landrace and is an example of how there are still many landraces to discover and study that could represent genetic resources of particular interest both in the scientific (for example plans for genetic improvement of crops) and socio-economic field. Conducting research which, like this, aims at researching and characterizing little known local cultivars should be a stimulus for universities and research centers which, besides contributing to broadening basic scientific knowledge, are actively working for the sustainable development of territories, interacting with various public and private parties.

This is even more true for those who are involved in supporting the development of mountain areas such as those in the Alps which, despite being rich in agrobiodiversity, are undergoing a gradual depopulation and abandonment of agricultural practices (and traditions) that are detrimental to agrobiodiversity. This work, the result of multidisciplinary research and cooperation between a research center (CRC Ge.S.-Di.Mont.), a territorial management body (Lombardy Region) and custodian farmers, represents the knowledge base that is required (and will be required) to launch actions for the conservation and promotion of "Grano Siberiano Valtellinese" that we hope will become a local raw material for the production of unique and quality healthy foods, therefore a resource for the economy of a mountain territory of the Alps and a further element of wealth for the Italian and European agri-food sector. We hope that further, similar research will be conducted in the future so as to counteract the loss of an immense genetic heritage (landraces) that could prove of fundamental importance for sustainable development, not just of mountain areas (Giorgi and Scheurer 2015). To counter this loss is also our duty from an ethical and moral point of view since landraces have represented the food of generations of those who worked the land (our ancestors) and have been handed down to us as a result of their hard work and immense sacrifices.

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#### **Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This research complies to the ethical rules applicable for this journal.

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