**Characterisation of bitter vetch (Vicia ervilia (L.) Willd) ecotypes: an ancient and promising legume**

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**Supplementary materials**

**Supplementary material 1**. Materials and methods

1. physico-chemical analysis of the soils

Soil samples were oven-dried at 60°C until constant weight to determine soil humidity (%), then grounded and passed through a 2 mm sieve to remove larger particles. The pH (water and KCl) was read with a standard calibrated pH meter in 2:1 distilled water and dry soil ratio(Jackson, 1958). The nitrogen was determined by mineralisation and distillation using the Kjeldahl method. A flame photometer analysed exchangeable potassium in 1 N ammonium acetate extract(Toth and Prince, 1949). Available phosphorus was analysed by the colourimetric method(Olsen *et al.*, 1954). The Electrical Conductivity (EC) was measured in the soil extract collected from the saturated soil paste by a conductivity meter(Wilcox, 1950). Total limestone (CaCO3) was measured by treating the sample with HCl(Nelson, 1983). Carbon was measured through dichromate oxidation and converted to Organic Matter (OM) by multiplying by a factor of 1.72(Walkley and Black, 1947). Soil texture was determined by using the standard Pipette method and wet sieving(Robinson, 1922).

1. Phenological assessment

On a whole plot basis, phenological observations were recorded every three days to determine days to start flowering (SF), days to full flowering (FF), and days to pod setting (PODS). Flowering duration (FDUR) was calculated through the difference between SF and PODS. The phenological stages were expressed in days and growing degree days (GDD) by considering the base temperature of 10°C(McMaster, 1997). They were determined by the following formula:

Accumulated GDD (°C) = Σ [ (Tmax + Tmin)/2]- Tb,

where Tmax is the daily maximum temperature (°C), Tmin is the daily minimum temperature (°C), and Tb is the base temperature (4°C as for *Vicia sativa* L.(Huang *et al.*, 2021)).

1. Morphological assessment

Five plants in each plot were randomly chosen for the agro-morphological characterisation at each phenological stage. Plant height (PH) was evaluated from the base to the tip of the plant. Stem diameter (SD) was measured at the third internode of each plant. Leaves number (LN), internodes number, and number of primary branches were counted on each plant. Leaflet number per leaf (LLN), leaf length (LL), and leaf width were measured from ten randomly selected leaves per plant. Five plants in each plot were randomly chosen at the maturity stage to characterise the number of pods and grains per plant (PODN and GPL) and the root length. The grains per pod (GPOD) and the pod length (PODL) were assessed from ten randomly selected pods per plant.

1. Agro-Morphological assessment

All the remaining plants were harvested to determine grain yield (GRY) on a dry matter (DM) basis. Thousand seed weight (TSW) was recorded as the weight of one thousand seeds sampled thrice from bulked seeds in each plot. The straw was measured as the dry weight of the remaining above-ground plant parts. The harvest index (HI) was calculated as the ratio of GRY to straw.

1. Bromatological analysis.

The parameters were evaluated according to AOAC (1990)to determine the content in DM by drying 5 g of the sample at 102°C until constant weight (method 934.01), ash by incinerating 5g of the sample at 550°C for 12h (method 942.05), and ether extract (EE) by extraction with diethyl ether in Soxhlet apparatus (method 963.15). The crude protein (CP) content was determined by multiplying the nitrogen content by 6.25, obtained after mineralisation with H2SO4 and distillation with NaOH using the Kjeldahl method (method 977.02). Fibre content (crude fibre (CF), neutral detergent fibre (NDF); acid detergent fibre (ADF), and acid detergent lignin (ADL)) were analysed using an ANKOM® 200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA), following the method of AOAC (1990) and Van Soest *et al.* (1991). The nitrogen-free extract (NFE) was estimated using the following formula:

NFE (% DM) = 100 − (EE + CP + CF + Ash).

*In vitro* enzymatic DM and OM digestibilities (IVEDMD and IVEOMD) were determined by the enzymatic method in a two-step method with first incubation of a 2% pepsin solution diluted in 0.1 N hydrochloric for 24h and second with solubilisation in a buffer solution containing 1 g L-1 cellulase, both at 40°C Huang(Aufrère and Michalet-Doreau, 1983). The metabolizable energy (ME; MJ kg-1 DM) was calculated using the equation(AOAC, 1990):

ME = 0.17 × IVEDMD - 2,

where IVEDMD is the enzymatic dry matter digestibility in % DM. *In vitro* true digestibility (IVTD) was determined by incubation of feed samples in filter bags in a Daisy II incubator ® (ANKOM Technology, Fairport, NY, USA)(Kowalski *et al.*, 2014). Artificial saliva in a 1:5 ratio was mixed with collected rumen fluid from a slaughterhouse and added to heat-sealed ANKOM F57 filter bags containing samples and incubated at 39.5°C for 48h. The in vitro enzymatic crude protein digestibility (IVECPD) was determined according to the procedure described byAufrère and Cartailler (1988). Briefly, 1.0 g of ground sample was added to 50 mL of enzyme solution (0.1 g protease per 1L of borate–phosphate buffer; pH 6.8). Then, the tubes were sealed and incubated at 40 °C for 24 h under permanent stirring. Subsequently, samples were filtered, and residual N content was analysed. The IVECPD was calculated according to the following equation:

IVECPD (%) = (N sample-N residue) / N sample × 100,

where Nsample represents the sample's nitrogen content, and Nresidue represents the nitrogen remaining after digestion.

1. Antioxidant activity

One gram of flour from ground grains was extracted with 70 mL of 70% ethanol. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined according to the procedure mentioned by Chaieb *et al.* (2011). Briefly, 100 μl of the extract was mixed with 100 μl of 0.1 mM DPPH in ethanol. The absorbance was read after a 30 min incubation in the dark against a blank at 517 nm at room temperature. The DPPH radical scavenging in percent (%) was calculated in the following way:

Inhibition (%) = [Ablank- (Aextract-Asample)]/Ablank × 100,

where Ablank was the absorbance of the control solution (containing only DPPH), Aextract was the absorbance for the plant extract in the presence of the DPPH solution, and Asample was the absorbance for the plant extract solution without DPPH solution. The ferric-reducing ability of plasma (FRAP) of the bitter vetch grain extract was determined according to the method described by Chaieb *et al.* (2011). Samples (20 μL of extract) in a 96-well microplate were mixed with 1.2 mL of freshly prepared FRAP reagent (10 mmol L-1 TPTZ [2,4,6-tri (2-pyridyl)-triazine] in 40 mmol L-1 HCl, 300 mmol L-1 acetate buffer, and 20 mmol L-1 FeCl3·6H2O, pH 3.6 in a:1:10:1 ratio). The solution was incubated in darkness at 25°C for 30 min. Absorbance was measured against a blank at 595 nm in a microplate reader. An aqueous solution of known ferrous sulfate concentrations was used for calibration, and the results were expressed in mg FeSO4 g-1 of dry sample. The quantification of total phenols was performed according to the procedure described by Makkar *et al.* (1993) using the Folin–Ciocalteu reagent. Briefly, 0.25 mL of the ethanolic extract was mixed with 0.25 mL of Folin-Ciocalteu reagent, 19.5 mL of distilled water, and 1 mL of Na2CO3 (5%). After vertexing the solution, the absorbance was read at 720 nm against a blank after one hour. Tannic acid (0-1 mg mL-1) was used to obtain the standard curve. Results were expressed as mg TAE g-1 DM of grains. In another centrifuge tube, non-tannic phenols were determined by mixing 3 mL of the extract with 300 mg of polyvinylpolypyrrolidone and 3 mL of demineralised water. The tube was vortexed, kept at 4°C for 15 minutes, centrifuged at 4°C at 3500 rpm for 10 minutes, and then read at 725 nm. Total tannins were calculated as the difference between non-tannic phenols and total phenols. Condensed tannins (CT) were assayed by Porter *et al.* (1985) method using butanol-HCl and reading the absorbance at 550 nm.

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Table S1: correlation coefficients of the correlation matrix of agro-morphological and bromatological traits of 17 cultivated Moroccan bitter vetch ecotypes.



Table S2: Correlation of the variables to the 3 dimensions in the principal components.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Dim1** | **Dim2** | **Dim3** |
| **Stem diameter** | 0.6 | -0.1 | -0.35 |
| **Plant height** | 0.78 | 0.31 | -0.27 |
| **Leaf length** | 0.77 | 0.23 | -0.03 |
| **Leaflet number** | 0.6 | 0.15 | -0.38 |
| **Leaf number** | 0.6 | 0.32 | -0.26 |
| **Pod length** | 0.51 | -0.14 | -0.33 |
| **Pod number** | 0.27 | -0.76 | 0.29 |
| **Grain per plant** | 0.33 | -0.62 | 0.22 |
| **Harvest index** | 0.58 | -0.18 | 0.47 |
| **Thousand seed weight** | 0.6 | -0.02 | -0.12 |
| **Grain yield** | 0.71 | -0.18 | 0.57 |
| **Acid detergent lignin** | -0.49 | -0.65 | -0.16 |
| **Metabolisable energy** | 0.11 | 0.59 | -0.14 |
| **Ash** | -0.42 | 0.62 | -0.03 |
| **Condensed tannins** | -0.36 | 0.34 | 0.37 |
| **FRAP** | 0.27 | 0.57 | 0.61 |
| **Phenols** | 0 | 0.63 | 0.62 |
| **Start of flowering** | -0.22 | 0.3 | -0.58 |