*Journal of Applied and Natural Science*
14(1), 68 - 72 (2022)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

*Research Article*

# Total and chloroplast proteins of the leaves of cultivated, population and wild sunflower varieties cultivated under thermic stress

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***Article Info***
https://doi.org/10.31018/jans.v14i1.3137
Received: October 30, 2021
Revised: February 3, 2022
Accepted: February 8, 2022

***How to Cite***
Ouazzani, C. (2022). Total and chloroplast proteins of the leaves of cultivated, population and wild sunflower varieties cultivated under thermic stress. *Journal of Applied and Natural Science*, 14(1), 68 - 72. https://doi.org/10.31018/jans.v14i1.3137

**Abstract**
Sunflower (*Helianthus annus L*) cultivation is affected by periods of severe drought that affect the growth and yield of sunflower crops. Wild sunflower species have brought many agronomically important traits to cultivated sunflower by quickly allowing them to undergo biotic and abiotic changes in their environment. Plants have developed several mechanisms that would enable them to tolerate high temperatures related to thermotolerance at the biochemical and metabolic levels. The present study aimed to highlight the variability of total and chloroplast proteins extracted from the wild and populations of sunflower leaves grown under different thermal stresses. The total proteins extracted from controlled and thermic stress leaves, characterized by SDS page were similar except polypeptides of molecular weight (MW) 42 kDa, 35 kDa, 31 kDa and 13.5 kDa which showed variability of sunflower varieties studied. Under thermic stress, the MW of chloroplast proteins was similar in the *H petiolaris fallax* and *H praecox runyonni* varieties. The protein markers of wild sunflower species revealed in response to thermal stresses contribute to the improvement of sunflowers in the face of environmental challenges.

**Keywords**: Proteins, Chloroplasts, *Helianthus* spp, Leaves, Sunflower, Thermic stress.

## INTRODUCTION

Supra-optimal temperatures and water stress are among the most damaging climate change factors for crop yields. Under thermic stress, plants have morphological adaptation mechanisms such as the vertical position of leaves and leaf winding along their major axis among grasses. Other morphological adaptation mechanisms include leaf hair production (pubescences) and waxy surfaces that reflect light, reduce energy, and produce small, heavily cut leaves that reduce the boundary air layer and allow maximum heat loss by convection or conduction (Najeeb *et al*., 2019).

In addition to these morphological adaptations, plants have developed several mechanisms that allow them to tolerate high temperatures, thermal dependence at the biochemical and metabolic level and thermal tolerance in relation to membrane stability induced by a gradual increase in temperature with respect to the production of proteins against thermal shock. The response to heat results in the production of specific proteins that are called “heat shock proteins” or HSP, a group of proteins whose expression is increased following stress, classified according to their molecular mass (kDa) (Park and Seo, 2015). Heat shock proteins are involved in signaling, translation, the host’s defense mechanisms, carbohydrate metabolism and amino acid metabolism (Magaji *et al*., 2014). Heat stress has significant effects on protein metabolism, including degradation of proteins, inhibition of protein accumulation, and induction of certain protein synthesis, depending on the level and duration of heat stress (Jacob *et al*., 2017; Rai and Agarwal, 2018).

Isoprene synthesis allows rapid membrane stabilization in leaves subjected to sudden increases in temperature. It can protect like carotenoids, the photosynthetic apparatus effects of strong lights (photoinhibition), reacting with singular oxygen formed under the action of exited chlorophyll (in triplet state). It can also react with OH radicals which form when hydrogen peroxide is produced in large quantities (Fini *et al*., 2017).

The genus *Helianthus* has 51 species, 14 annuals and 37 perennials. Wild sunflower species have brought many important agronomic traits to cultivated sunflower. Wild species are adapted to a wide range of habitats and have variability in most biotic and abiotic char-

acteristics (Qiu *et al*., 2018). Introgression of cultivated sunflower by wild material is a method of selection of sunflowers in the face of drought resistance.

Wild Helianthus species exhibit genetic diversity that increases sunflower resistance to abiotic stress, particularly with *H. argophyllus* and *H*. *paradoxus*, *H. deserticola*, *H. hirsutus*, *H. maximiliani* and *H. tuberosus* (Kaya, 2017; Skoric, 2016).

Cultivation of sunflower in Morocco is limited in regions with high temperatures. The major effect of high temperatures on sunflower cultivation is a decrease in organ fertility. The composition of the seeds is affected by the protein and oil content. These reductions in the quality of the composition of the seeds have repercussions on industrial quality. The effect of thermic stress is on the foliage or reproductive organs (burns).

The present work aimed to compare the genetic performance diversity of traditionally cultivated populations and hybrid varieties. The study proposed to demonstrate the variability of total and chloroplast proteins extracted from leaves of hybrid wild sunflower and population leaves controlled and subjected to heat stress.

The characterization of marker polypeptides in plants subjected to thermal stress constituted molecular markers for selecting plants facing the suitability of growing conditions under high temperature conditions and the search for the mechanisms involved.

## MATERIALS AND METHODS

### Materials

Fourteen varieties of sunflower seeds were procured from the French (National Institute for Agricultural Research), (INRA) genetics research unit and plant improvement Montpelier. They are made up of cultivated hybrids, populations and wild varieties. The list of which is shown in (Table 1). Several trials of sunflower cultivation under the effect of heat stress were carried out to select sunflower varieties showing good suitability for cultivation in a high temperature environment. Agronomic parameters such as leaf density and plant height were determined in the different varieties of sunflower conditioned to thermal stress.

### Methods

#### Total protein extraction

Leaves (0.2 g) were taken from the planting plots of the different varieties of sunflower in greenhouses. After rinsing the leaves with 0,01% (v/v) NaOCl added, 2 ml of the buffer solution (62.5 mM Tris – HCl, pH 6.8, 2% SDS, 10% glycerol) was added at 4°C. After grinding in a mortar for 3 min, the ground extract was boiled for 3 min at 100°C. After centrifugation for 10 min at 5000 g, the supernatants were stored in different aliquots at -20°C.

#### Isolation of chloroplasts from sunflower leaves

Leaves (0,5 g) were taken from sunflower plants grown in greenhouses in a thermal environment. After rinsing the leaves with 0,01% (v/v) NaOCl, the leaves were crushed in 2,5 ml of the buffer solution containing 0.1 M Tris, 0.2% albumin, 0.1% beta mercaptoethanol and 1% PVP. After removal of cell debris for 10 min at 500 g at 4°C, the supernatants containing the chloroplast pellets were sedimented at 2600 g for 15 min at 4°C. The chloroplast pellets were dissolved in grinding buffer and stored at -20°C.

#### Electrophoretic analysis

The chloroplast pellets solubilized in buffer 0.1 M Tris pH 8, 10% glycerol, 2% SDS, and 0.1% β mercaptoethanol were heated for 5 min at 100°C. After centrifugation at 8000 rpm pendant for 5 min at 4°C, cell debris was removed and the supernatants were stored for electrophoretic analysis at -20°C. Electrophoresis was carried out on polyacrylamide gels in the presence of

**Table 1**. List of populations, wild and cultivated sunflower varieties

| Code variety | Origin | Characteristic |
|---|---|---|
| 125 | Moroccan | Cultivated population |
| 62=HA89 | Vniimk 8931 Russe | Cultivated population |
| 63= 2603 | Morrocan | Cultivated population |
| 83HR4 | Vniimk 6540, GS09, RHA | Population |
| 89B1 | H praecox Runyonni | Wild species |
| 89B2 | H niveus canescens | Wild species |
| 91T608 | H niveus tephrodes | Wild species |
| 92B6 | Argo F2-7P | Wild species |
| 92T571/2 | H petiolaris Fallax | Wild species |
| 88-301-5 | H debilis tardiflorus | Wild species |
| 89-1368-3 | H debilis silvest | Wild species |
| 92-239-1 | H niveus tephrodes | Wild species |
| 92-564-5 | H petiolaris fallax | Wild species |
| 93-170-6 | H praecox praecox | Wild species |

SDS (Laemlli and Favre 1973).

## RESULTS AND DISCUSSION

### Total proteins responses thermic stress

Electrophoretic analysis (Fig. 1) revealed similarity of MW (molecular weight) between 66 kDa and 14 kDa of polypeptides in different varieties of sunflower except the polypeptides of MW 42 kDa, 31 kDa and 13.5 kDa which showed a variability. The polypeptide of MW 42 kDa showed a decrease in colouration intensity in the varieties of Moroccan population (2603) and Vniimk 6540 under conditions of heat stress. The 31 kDa molecular weight polypeptide exhibited a decrease in staining intensity in the Moroccan population (125), Moroccan population (2603) and *H niveus canescens* varieties under heat stress conditions compared to control conditions. The electrophoretic profiles of polypeptides of the *H praecox* variety were similar under heat stress conditions compared to control conditions. The polypeptide of MW 13.5 kDa has a decrease in colouration intensity under heat stress conditions compared to control conditions in the Moroccan population (2603) (125) varieties, Vniimk 6540 RHA, *H niveus canescens*, *Argo F2- 7 p*, *H debilis tardiflorus.* This polypeptide was absent in the variety *H niveus Tephrode*. Under conditions of heat stress, this polypeptide is absent in the Moroccan population (2603) and Vniimk RHA 6540 varieties. The decreased protein concentration could be linked to reduced protein synthesis during heat stress. The effects of heat stress lead to cell degradation and cell death. The action of heat leads to the denaturation of membrane proteins, the melting of membrane lipids and the loss of cellular content (Kaushal *et al.,* 2016). The work described by Slattery and Ort (2019) and Wijewardene *et al*., (2021) revealed the sensitivity of Rubisco, a major enzyme in photosynthesis to temperature and the differential expression of the small subunit (SSU) isoforms associated with Rubisco plasticity in changing environments. Yamamoto (2016) revealed the degradation of the D1 protein, constituting the core complex of photosystem II (PS) by ROS produced during an increase in temperature.

### Chloroplastics proteins stress thermique response

Electrophoretic analysis of chloroplast proteins (Fig. 2) revealed polypeptides of molecular weights between 43.5 kDa and 14 kDa under normal conditions in the different varieties studied, population Moroccan (2603), *H niveus Tephrodes*, *H petiolaris fallax*, Vniimk 8931, *H praecox fallax*, *H praecox Runiyonnii* except the polypeptide of molecular weight 38 kDa present in the variety *H petiolaris*. Under stress thermic conditions, the molecular weights are preserved in all the varieties studied with the exception of the 38 kDa polypeptide which is present in the variétés *H niveus tephrodes* and H *petiolaris fallax*. The electrophoretic profiles of the varieties H *petiolaris fallax* and H praecox *runyonni* are conserved under control and thermic stress. The works described by Seiler (2017) have revealed specific resistance genes cytoplasmic to abiotic stress diseases in the variety H. *petiolaris* which is highly adapted to deserts. The species H *argophyllus* and *H niveus* constitute species with root and leaf characteristics adapted to desert conditions (Bowsher, 2016).
Polypeptides extracted from chloroplasts of the variety

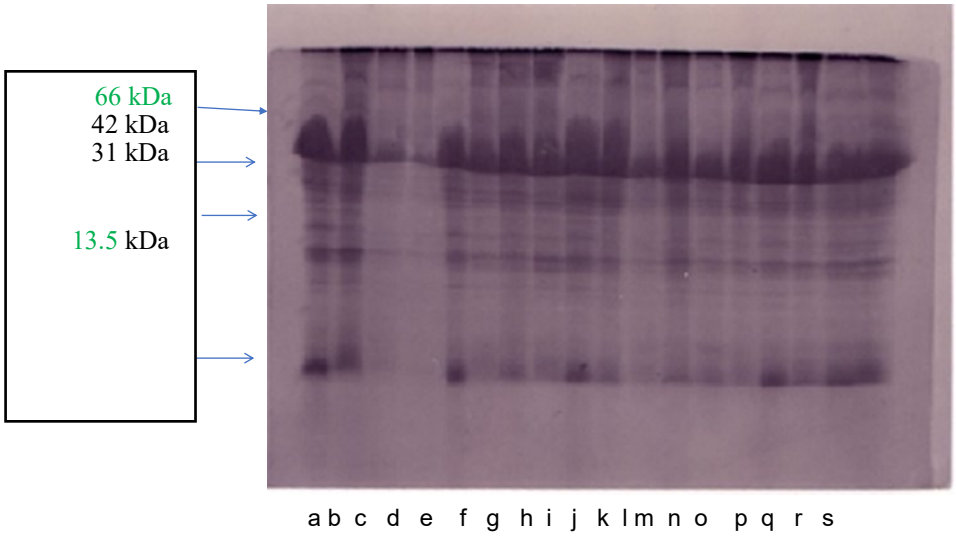


**Fig. 1**. *Polyacrylamide gel electrophoresis (12%) in the presence of SDS of total protein extracted from sunflower leaves grown under controlled conditions (1) and heat stress (2). a– molecular weight standard. b (1), c (2) Moroccan population* (125)*, d (1), e (2) Vniimk 8931 Russe, f (1), g (2) Moroccan population* (2603)*, h (1), i (2) Vniimk 6540, 6509 RHA, j (1), k (2) H niveus canescens, l (1), m (2) H niveus Tephrode, n (1), o (2) Argophyllus F27P* (92B6)*, p (1), q (2) H debilis Tardiflorus, r (1), s (2) H praecox praecox.*

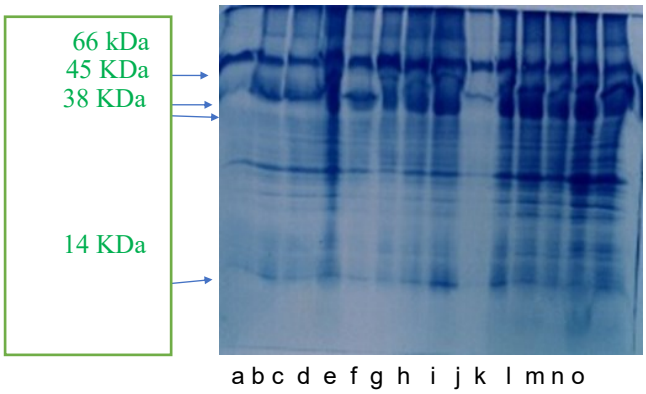


**Fig. 2** *Polyacrylamide gel electrophoresis 12% in the presence of SDS of chloroplast protein extracted from sunflower leaves grown under controlled conditions (1) and heat stress (2). a molecular weight standard, b (1), c (2), H praecox, d (1), e (2), Morrocan population (2603), f (1), g (2), H niveus tephrodes, h (1), i (2), H petiolaris fallax, j (1), k (2) Vniimk 8931 Russe, l (1), m (2) H petiolaris fallax, n (1), o (2) H praecox runyonni.*

praecox are similar under normal and heat stress conditions. Under stress, remodeling and intensive degradation of chloroplast proteins can occur releasing a large number of endogenous proteins (Hu *et al.,* 2020). Photosynthetic electron transport and ATP synthesis are greatly affected if PSII suffers from severe thermal damage (Wang et al., 2018). A recent study revealed that reactive singlet oxygen, $^1O_2$, is produced and can damage PSII reaction center proteins, which in turn induces the PSII repair cycle (Dogra and Kim 2019). Peptides derived from chloroplast proteins may function as regulators of the plant's responses to abiotic stress and manifest via increased production of proteins involved in protein quality control processes (heat shock proteins, chaperones, proteases and reactive oxygen species (ROS) detoxifiers (Mamaeva *et al*., 2020).

Electrophoretic analysis of chloroplast (Fig. 3) proteins extracted from sunflower leaves grown under controlled conditions revealed polypeptides of molecular weights between 45 kDa and 14 kDa. The varieties *H debilis tardif* and *H debilis silvest* showed similar electrophoretic profiles under normal conditions compared to those under heat stress. The varieties H *debilis Tephrodes*, *H Argo* and *H niveus canescens* showed a decrease in the staining intensity of the electrophoretic profile conditions compared to that of normal conditions. The variety Vniimk RHA exhibited a polypeptide of molecular weight 35 kDa under thermic stress. This polypeptide is absent in the varieties *H debilis tardif, H niveus tephodes*, *H debilis silvest*, Argo F27P, H *niveus canescens* and the Moroccan population (125). Bernfur *et al.,* (2017) identified low molecular weight HS proteins from *Arabidopsis thaliana* some of which are transported in chloroplasts associated with thylakoid membranes in heat stressed plants. Zach and Wataru (2014) revealed the influence of high temperature and light on the induction of degradation and aggregation of chloroplast proteins resulting in the activation of plastid proteases that rapidly degrade aberrant turnover proteins. The work described by (Wang *et al.,* 2018) revealed metabolic reprogramming in response to heat stress in higher plants, which included chlorophyll degradation, generation of reactive oxygen species (ROS) antioxidant defense and protein renewal.

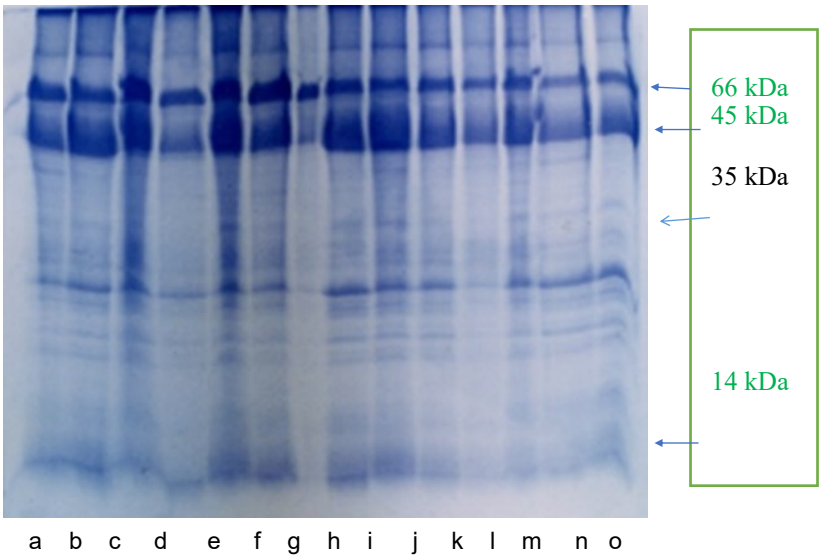


**Fig. 3.** *Polyacrylamide gel electrophoresis (12%) in the presence of SDS of chloroplast protein extracted from sunflower leaves grown under controlled conditions (1) and heat stress (2). a molecular weight standard, b (1) c (2) H debilis tardif, d (1) e (2) H niveus tephrodes, f (1) g (2) H debilis silvest, h (1) i (2) Vniimk RHA, j (1) k (2) Argo F27, l (1), m (2) H niveus canescens, n (1), o (2) Moroccan population (125).*

## Conclusion

The electrophoretic profiles of total and chloroplast proteins were similar in different wild and cultivated sunflower varieties cultivated under controlled conditions. Under thermal stress conditions the response to thermal shocks is expressed by the decrease in protein concentration, induction and absence of proteins in the chloroplast organelles in sunflower leaves.

Studies based on chloroplast protein purification, charge and sequence will highlight genetic variability in wild and cultivated species related to adaptation to high temperatures.

Identification of protein molecular markers contributes to improving the properties of photosynthetic complexes, antioxidant defence mechanisms and reprogramming metabolism in response to heat stress. The selection of sunflower varieties resistant to high temperatures contributes to the improvement of production, adapting the material to local climatic conditions

### Conflict of interest

The author declares that he has no conflict of interests.

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