# Electrophoresis of Rape Seed Extracts

V3 11/15

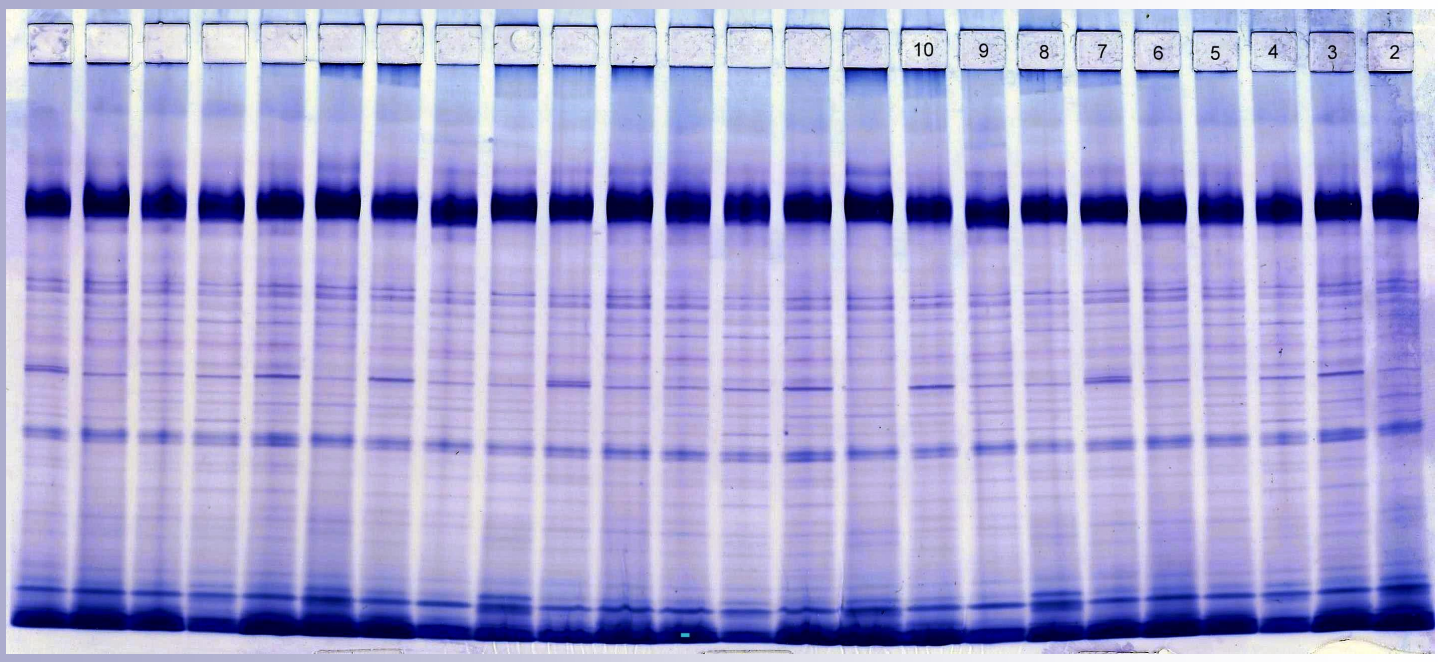


**Fig.1:** Extracts of 9 different rape varieties running in native cathodal elctrophoresis. Hot Coomassie staining.

***General**:* Two major techniques exist for analysis of complex protein mixtures under native conditions, the most common being Isoelectric Focusing (IEF) and native acrylamide gel electrophoresis.

Isoelectric focusing is based around the formation of a pH gradient typically using carrier ampholytes. IEF however, whilst being a high resolution method does not suit all applications as the carrier ampholytes may have undesirable effect on the proteins to be studied. Some enzymes can be affected and some membrane-protein complexes become unstable during the IEF procedure.
Above all the IEF of rape seed extracts give nice bands but with no discrimination between the varieties. See figure 6 on page 4.

Therefore the alternative of cathodal native acrylamide gel electrophoresis provides the solution. In general you will find the gel electrophoresis and the staining both quick and convenient. See figure 1, above.

**Hardware**

| | |
|---|---|
| flatbed IEF professional | edc-ief-2836 |
| Staining Tray Normal | edc-wm-n1 |
| DryPool Combi | edc-me-d |

**Consumables**

| | |
|---|---|
| DryGel Elpho 52S (à 5 µl) 4 gels | edc-411*3* |
| Protein Buffer Kit Cathodic | edc-5010 |

Rehydration & Electrode Buffers, Electrode Strips, Drying Cardboards, preserving sheets.

**Method:**

Extraction Buffer

| | |
|---|---|
| Rehydration buffer | 9 ml |
| Chloroethanol | 500 µl |
| Trimethylurea | 500 µl |
| Triton 10% | 30 µl |

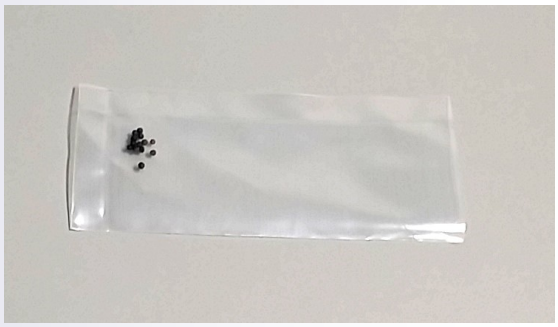

**Fig.2:** Melting seeds in a bag #1

Protein Extraction

1. Weight ~ 75 mg rape seeds (~10-12 grains)
2. Melt them in a plastic bag (fig.2 and 3)
3. Crash the seeds with a hammer
4. Add 500 µl $H_2O$, 10 min ultrasonic treatment
5. Centrifuge 5 min 5000 upm
6. Discard the supernatant
7. Add 800 µl Extraction Buffer
8. 25 min ultrasonic treatment
9. 10 min 57° C
9. Centrifuge 5 min 5000 upm

Final volume is ~ 500 µL

**Fig.3:** Melting seeds in a bag #2

**Fig.4:** Crashing the seeds with a hammer

**DryGel Treatment**

Weigh in 3 g urea in a 50 ml test tube and fill up with the rehydration buffer.

For the handling of the DryGel´s rehydration in the DryPool Combi (fig.3), see the manual coming with the buffer-system.

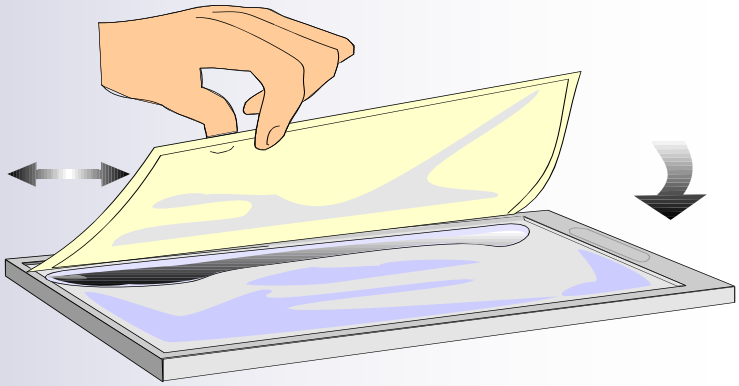

***Fig.5:*** Rehydration of a DryGel in the DryPool Combi

**The Run**

(15°C)

| | | | |
|---|---|---|---|
| 500 V | 10 mA | 10 W | 20 min |
| 900 V | 20 mA | 20 W | 30 min |
| 900 V | 25 mA | 25 W | 30 min |

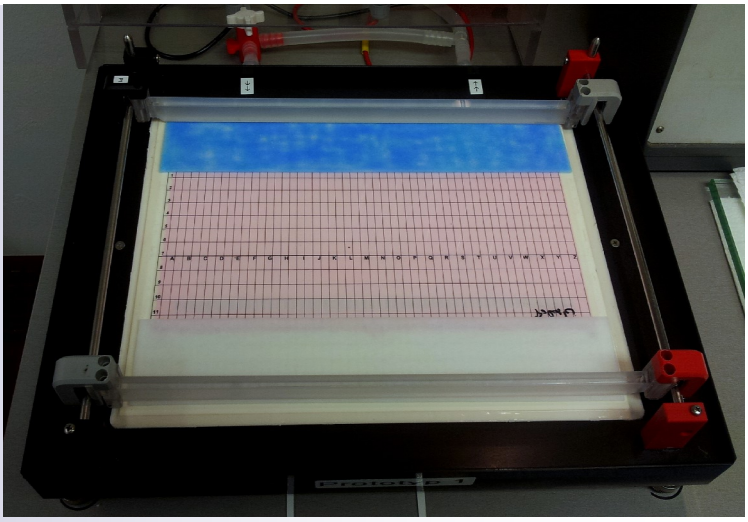

***Fig.6:*** Horizontal chamber: flatbed professional in cathodic direction

**Staining**

1. Hot Coomassie-Staining as general protein staining
General protein staining with Coomassie R-350 in hot acetic acid, see fig 5.
This hot Coomassie-staining is staining and fixing simultanuously!
The acetic acid for staining and destaining can be of technical quality.

*Staining solution:*
0.02% (w/v) Coomassie R-350 (GE 17-0518-01)
1 tablet (corresponds to 0.4 g dye substance) in 12.5% acetic acid. (Use fresh solutions only!)

*Destaining solution:*
12.5% acetic acid

*Impregnating solution:*
5% (v/v) glycerol

*Staining programme*:
30 min fresh staining solution at 60°C (exhauster) while stirring, see fig 5. Use EDC's staining tray!

3 x 20 min destaining solution in a tray on a rocking platform.

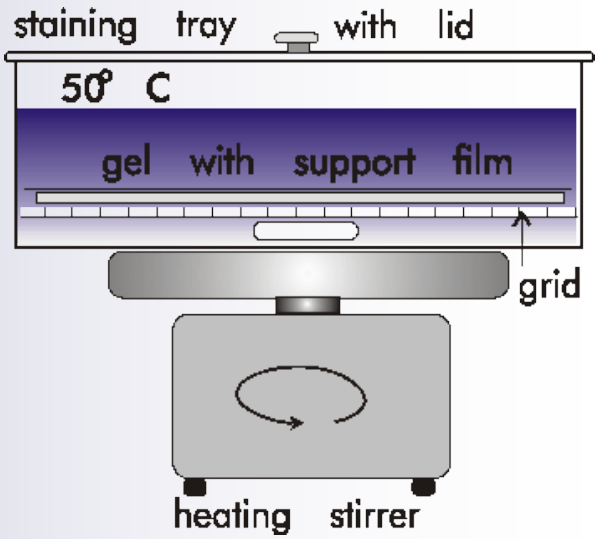


***Fig 5:*** Hot Coomassie-staining

20 min impregnating solution (tray).
Optimal staining can be achieved when the gel is placed in the first destaining solution overnight at ambient temperature.

## 2. Staining denaturing IEF with Blakesley / Roti Blue

Based on „Blakesley-Staining": ANALYTICAL BIOCHEMISTRY 82, 580-582 (1977)

A five fold concentrate staining solution can be purchased from Roth (Karlsruhe): 1 liter **Roti-Blue** (#A152.1). Sensitivity is 3-5 fold higher than other Coomassie-stainings.

Recipe and procedure for urea-containing IEF-gels:
*(double volumes for large sized gels)*

| | | | |
|---|---|---|---|
| fixing | 100 ml $H_2O$ | + 12.5g TCA | 30 min |
| washing | 70 ml $H_2O$ + 30 ml MeOH | + 1 ml Phosphoric acid | 15 min |
| staining | mix: 60 ml $H_2O$ + 40 ml MeOH | stir in: 20 ml Roti-Blue | 3h-overnight |
| washing | 75 ml $H_2O$ + 25 ml MeOH | - - - | 5 min |
| preserving | 3% Glycerol | - - - | 20 min |

Abbreviations: TCA = Trichloroacetic acid, MeOH = Methanol

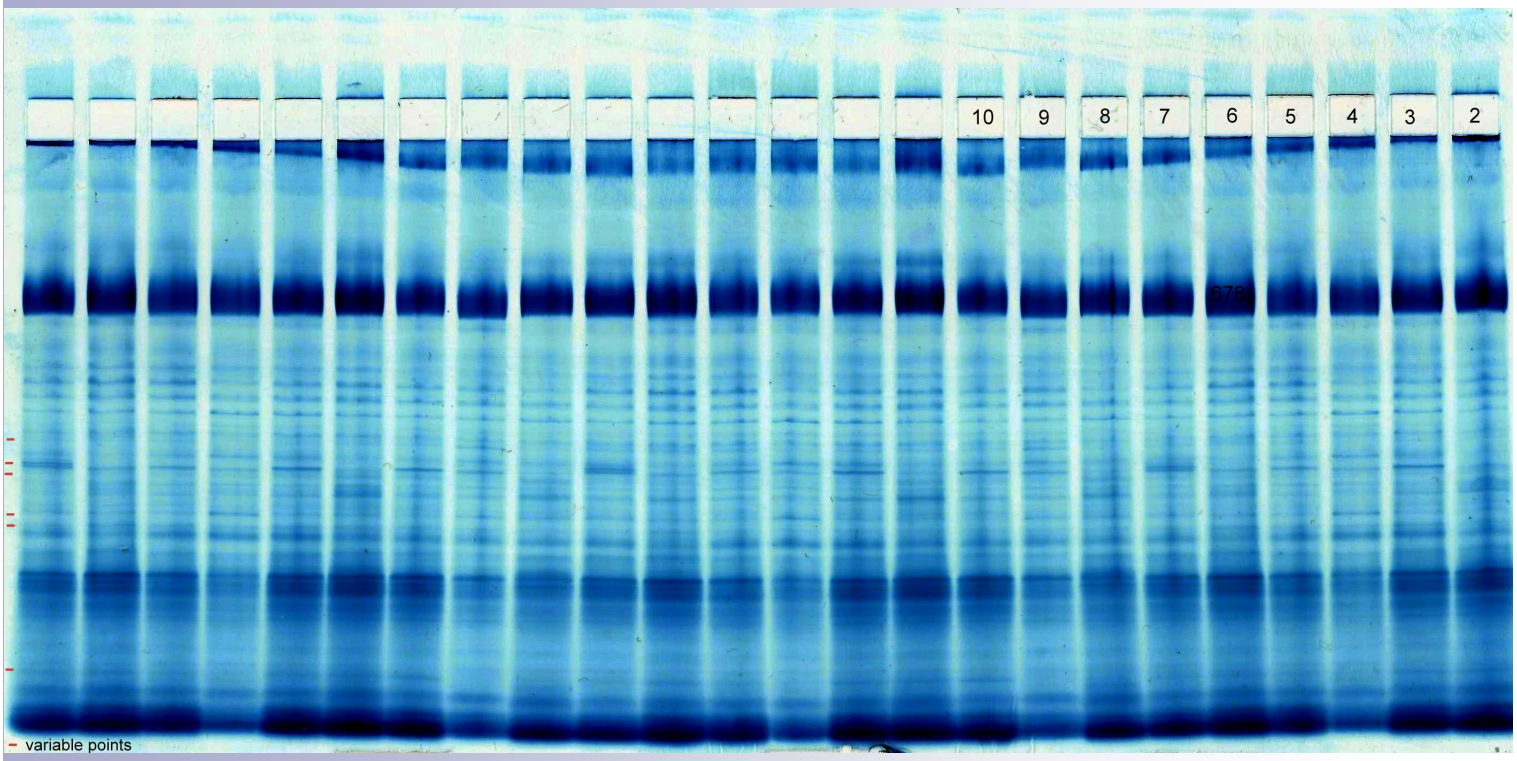


***Fig 6**:* native cathodic electrophoresis of 9 different rape varieties followed by Rothi-Blue staining

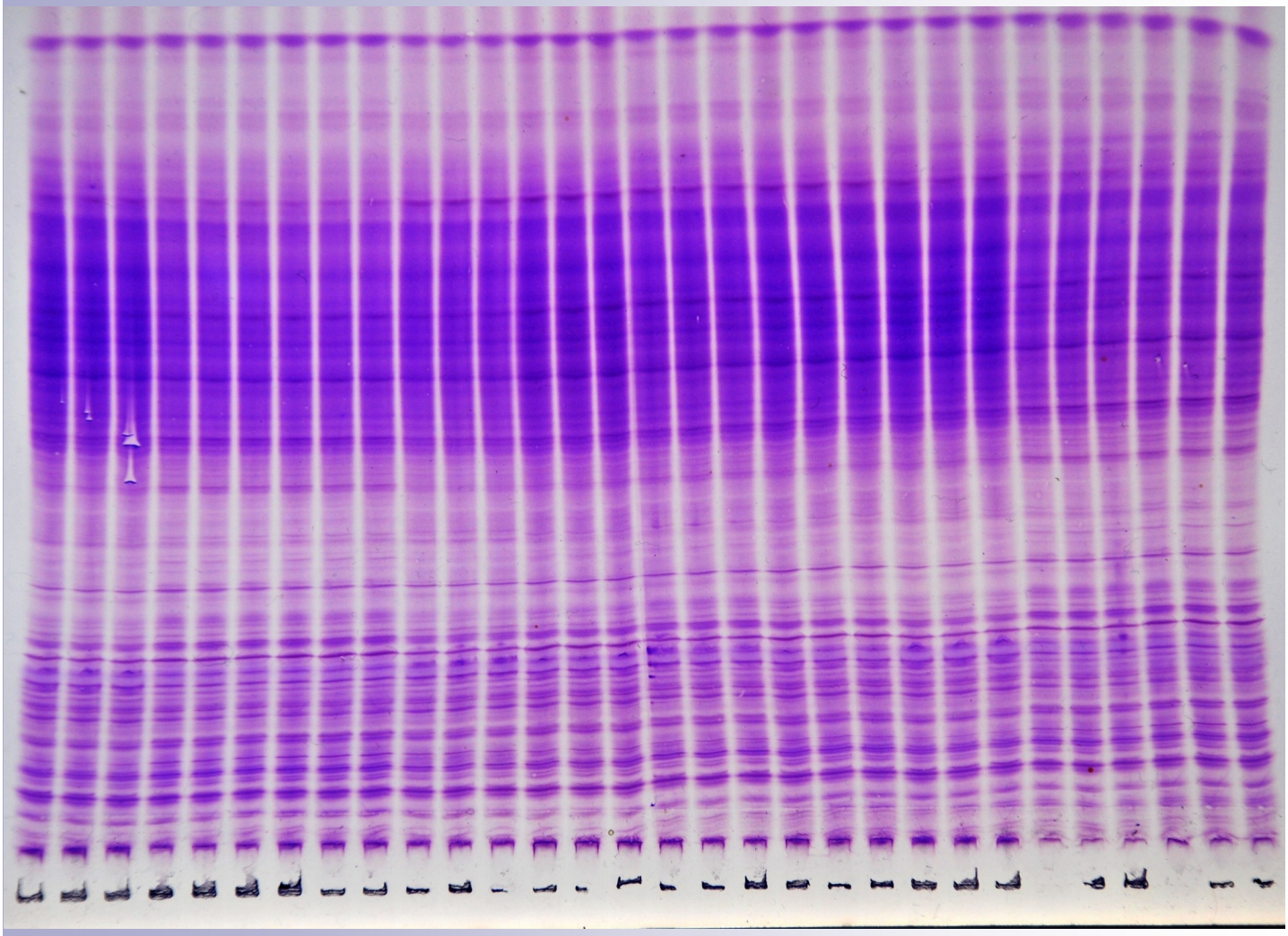

***Fig 7:*** IEF of rape varieties. No discrimination possible!