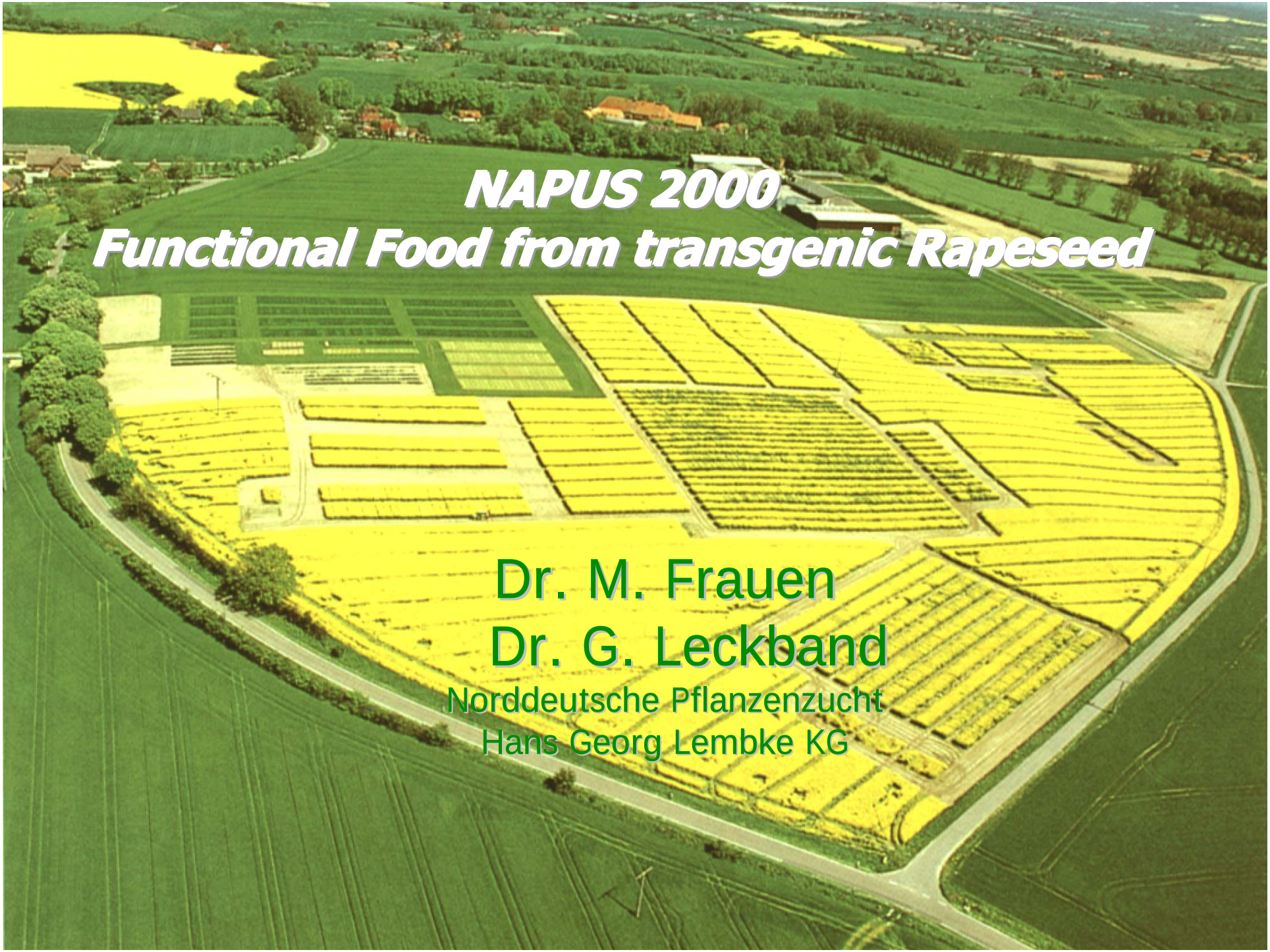


NAPUS 2000
Functional Food from transgenic Rapeseed

Dr. M. Frauen
Dr. G. Leckband
Norddeutsche Pflanzenzucht
Hans Georg Lembke KG

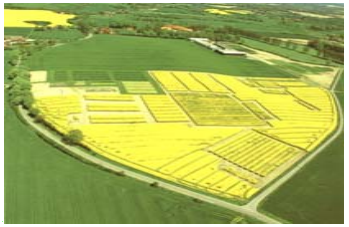
NAPUS 2000
Functional food from transgenic rapeseed

- **Leading Project Initiative of the German Federal Ministry of Education and Research** „Nutrition – modern processes for food production“
- Optimum use of the **whole** rapeseed kernel with improved quality for healthy food and as functional food
- Combine modern methods of genetic engineering with classical plant breeding to develop new varieties
- 20 partners from science, private plant breeding and industry

- Duration of sub-projects: between 2 – 5 years
- Duration of the entire project: October 1999 – November 2005
- Financial volume: 20.5 Mio. €
- Support volume: 13.6 Mio. €

Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, 24363 Holtsee
Dr. Martin Frauen, Prof. Dr. Wolfgang Friedt;
Dr. Gunhild Leckband

NAPUS 2000 – Thematic groups



Neutral lipids / LCPUFA

About fish and fatty acids

Optimising the fatty acid pattern of rapeseed

Tocopherol

About vitamines and oils

Accumulation of vitamin E in rapeseed oil

Reveratrol

About rapeseed, wine and the french paradoxon

Integration of resveratrol in rapeseed oil

Protein

About egg white, bitter compounds and yellow seed

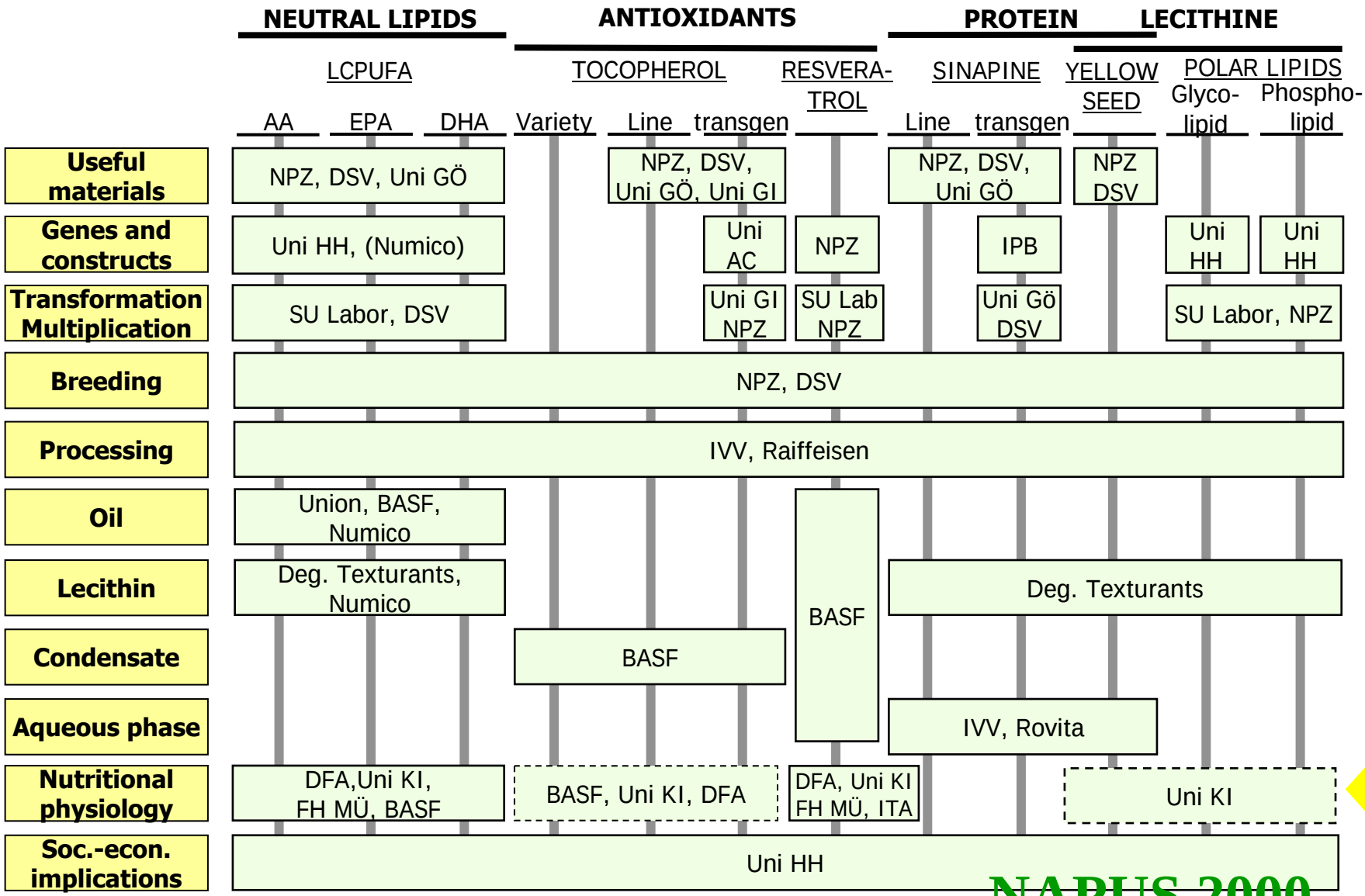
Use of rapeseed protein for nutrition

Polarlipids / Lecithin

About emulgators and classical breeding

Use of rapeseed lecithine as emulgator

NAPUS 2000 - interdisciplinary Network



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Subproject LCPUFA:
About Fish and Fatty acids

LCPUFA – The vision

Background:
Long-chained, poly-unsaturated fatty acids (LCPUFAs) are valuable for the human nutrition especially in terms of prevention and therapy of coronary heart diseases.

- Expression of LCPUFAs in rapeseed und linseed
- Use of plant-derived LCPUFA-oils as high value speciality oil and for the production of high concentrated supplements
- Development of a sustainable and ecologically useful alternative to present non-sustainable sources (seafood and algae)

Note:
This topic is of **world wide interest**,
The patent situation is complex
And:
Only a transgenic approach is possible!

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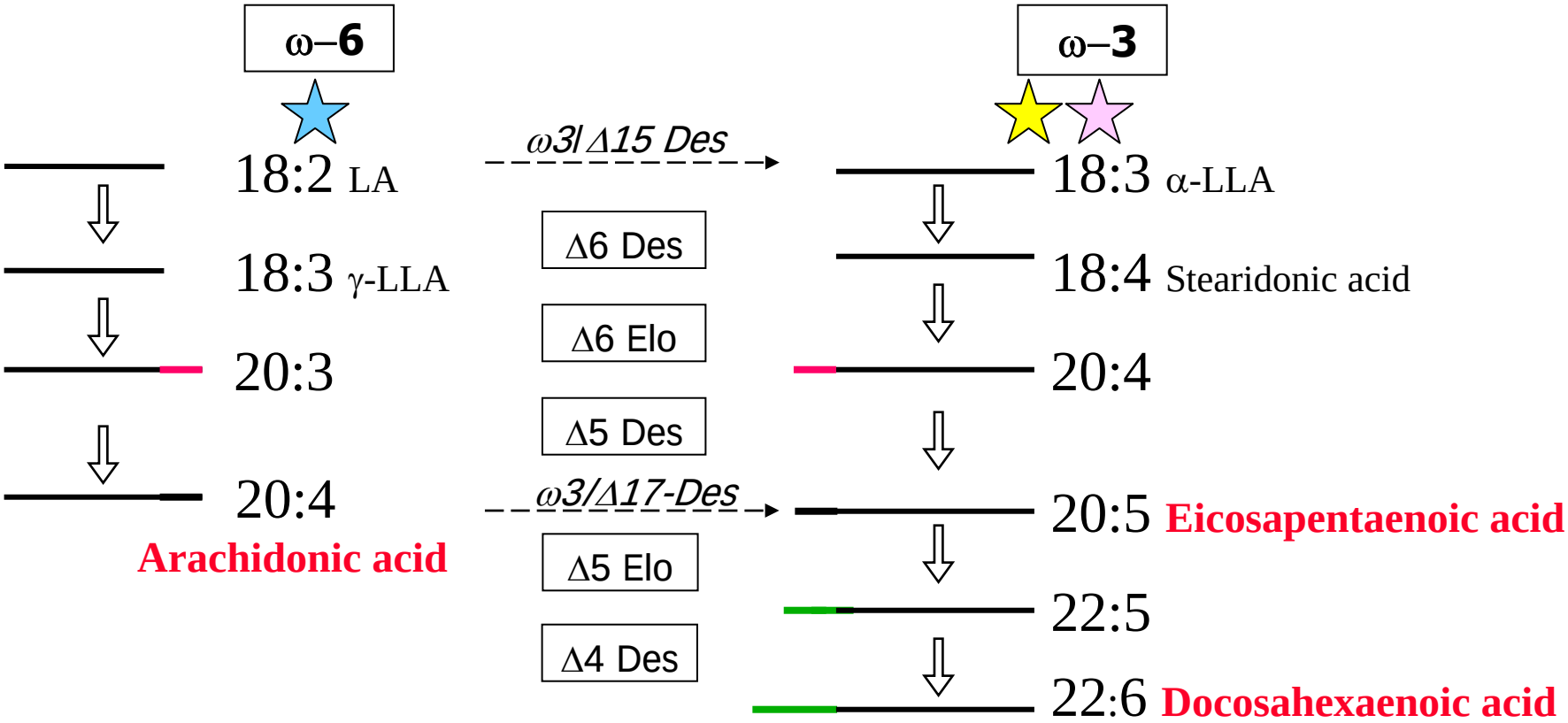
LCPUFA – The Strategy



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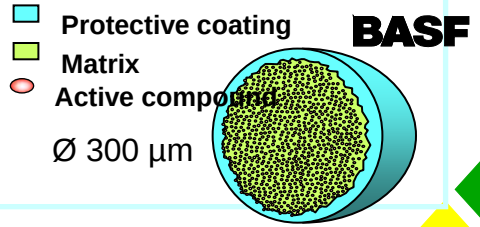
LCPUFA – The Status

	ARA	EPA	DHA
Useful materials	NPZ, DSV, Uni GÖ		
Genes and constructs	**Uni HH, Numico**		
Transformation Mulitplication	**SU Labor, DSV/NPZ**		
Breeding	**NPZ, DSV**		
Processing	IVV, Raiffeisen		
Oil	**Unilever, BASF,** Numico		
Lecithine	Deg. Text., Numico		
Condensate			
Aqueous Phase			
Nutritional physiology	**DFA, Uni Ki, FH Mü, Unilever, BASF**		
Soc.-econ. implications	Uni HH		

- All genes for LCPUFA-synthesis available
- Production of the worldwide first transgenic LCPUFA-Linseed plants containing more than 2% ARA and 1.5% EPA. Proof of concept → commercial implementation
- Description of alternative ways of synthesis aiming to increase efficiency

- Identification of classical breeding materials

- Trials for processing + formulation
  - Durability of oils
  - Emulsions
  - Beadlets



Nutritional physiology study
- Two studies each in animals and humans

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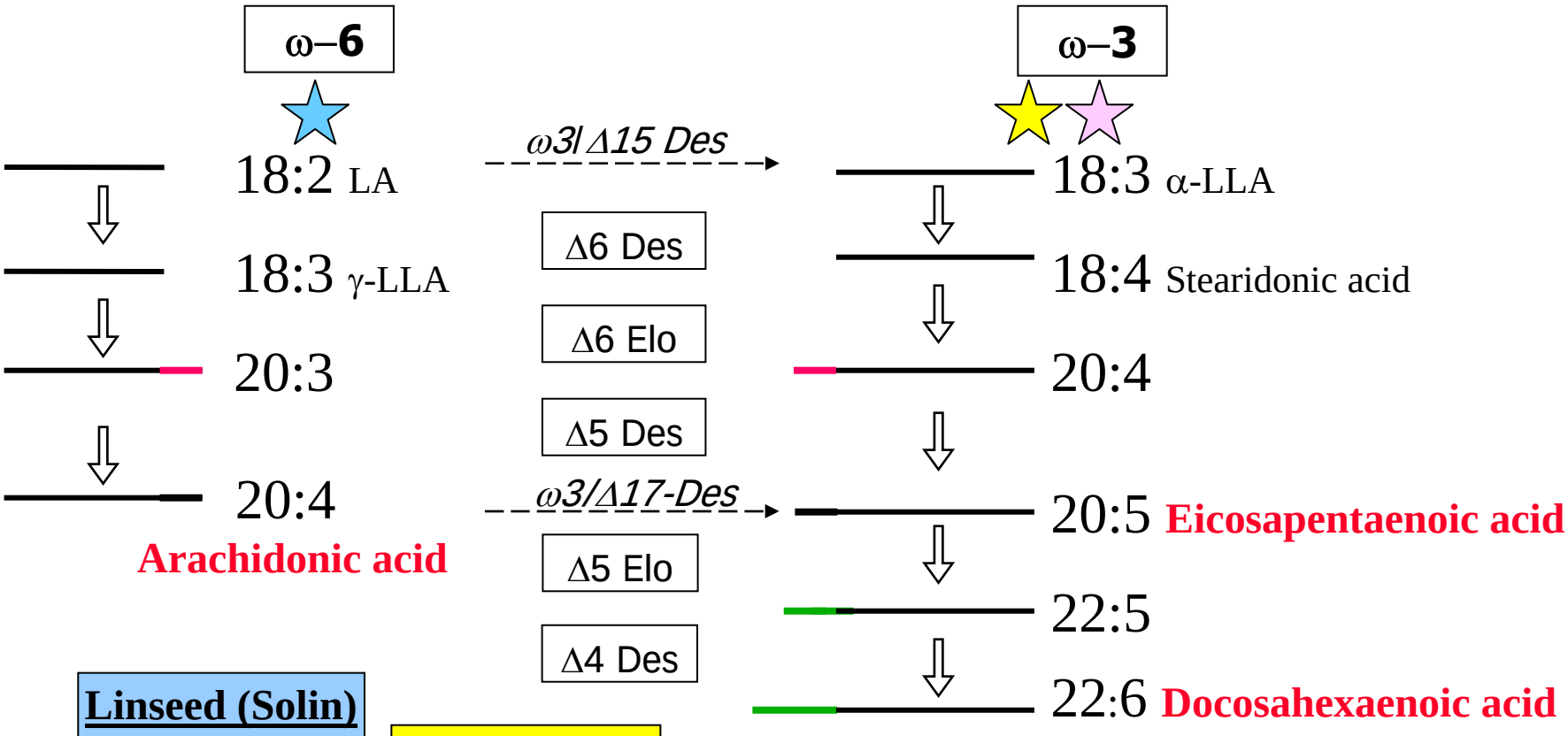
LCPUFA – The Strategy



Linseed (Solin)

15% 18:1
>**70**% 18:2
< 2% 18:3

HiLLA-Rapeseed

50% 18:1
23% 18:2
> **24**% 18:3

Linseed (conv.)

18% 18:1
15% 18:2
51% 18:3

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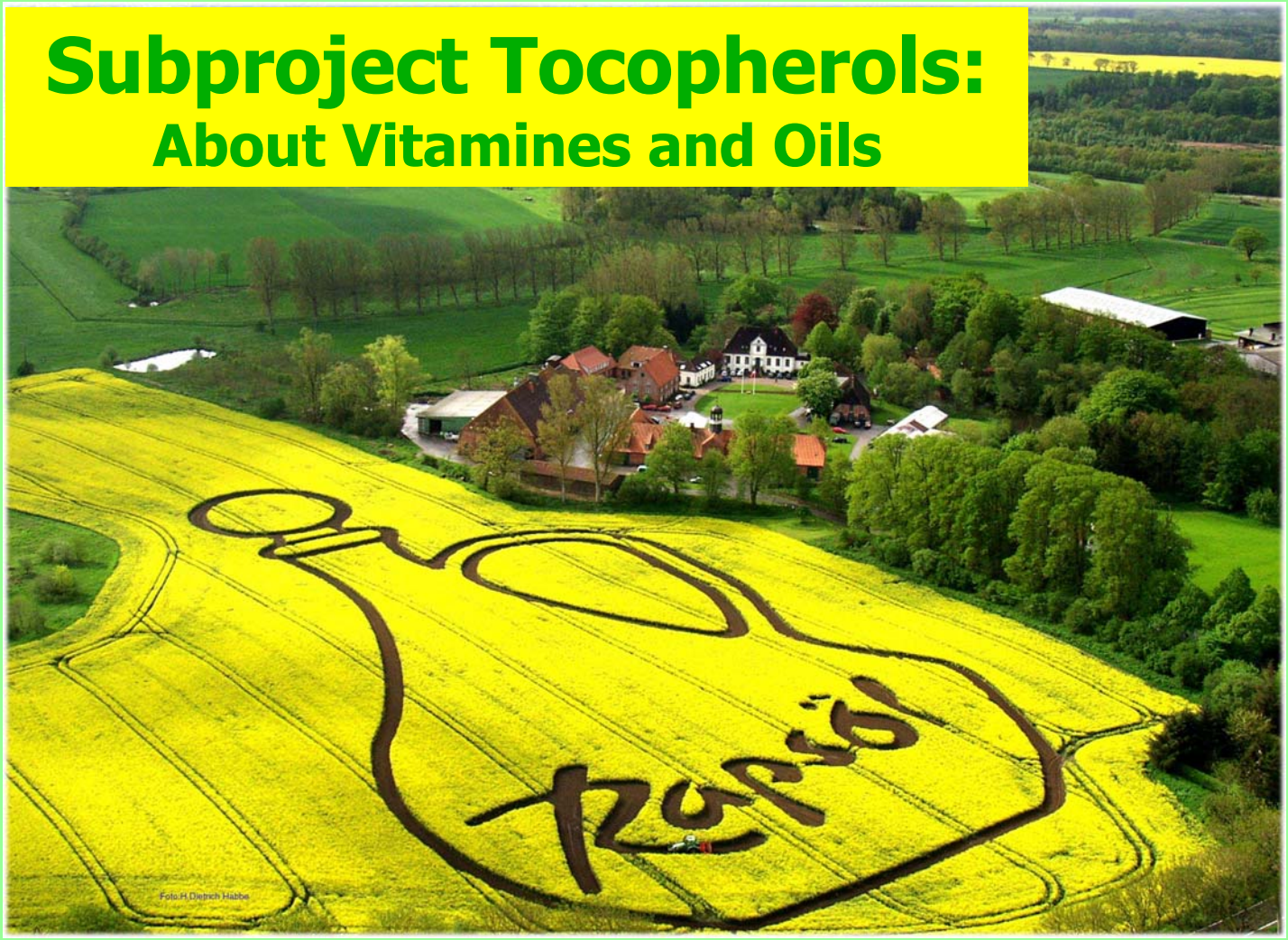
Subproject Tocopherols:
About Vitamines and Oils



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Tocopherol – The Vision

Background:

Vitamin E is a highly effective substance, it has a large antioxidative potential for prevention

Accumulation of Tocopherols in rapeseed

- Increasing the nutritional quality of rapeseed oil by higher α-tocopherol-content
- Improving of the oil stability, resp. stabilisation of LCPUFAs in rapeseed oil by higher γ-tocopherol content
- Improving the extraction of tocopherols from the condensate for further processing as supplements

Tocopherol – The Strategy

Parallel approach by classical and transgenic breeding

- **Transgenic approach**
  - Integration of additional enzymes for the Vitamin E-metabolism in rapeseed:
    1. Hydroxyphenylpyruvate-Dioxygenase (HPPD)
    2. Prenyltransferase
    3. Tocopherolcyclase
- **Classical breeding**
  - Selection of high Tocopherol rapeseed lines
  - Further breeding work for the development of conventional varieties
- **Processing**
  - Optimizing of extraction and processing methods

Tocopherol – The Status

	Variety	Line	transgene
Useful materials		NPZ, DSV, Uni GÖ, Uni GI	
Genes and constructs			**Uni AC**
Transformation Mulitplication			**Uni GI NPZ**
Breeding	**NPZ, DSV, Uni GI, Uni GOE**		
Processing	**IVV, Raiffeisen**		
Oil	**Unilever**, **BASF,** Numico		
Lecithin			
Condensat	**BASF**		
Aqueous phase			
Nutritional physiology	BASF, **Uni KI**, **DFA**		
Soc.-econ. implications	Uni HH		

- Creating transgenic plants with increased Tocopherol content:
  - Increase by **80%** to max. content of **1359 ppm** total TOC in the oil

- Analysis of variance for Tocopherol content in classical breeding material (200 to 1200 ppm)
- Selection of classical high TOC lines **(> 1000 ppm** total TOC in the oil)
- Studies on the inheritance of Tocopherol content

- Processing experiments
  - Back tracing of TOC during oil extraction process
  - Optimization of processing parameters / seed quality

- Experiments about formulations
  - Emulsions/Beadlets
  - Isolation of Vit. E by steam extraction

- Studies on the antioxidative potential in rapeseed

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Subproject Resveratrol:
About Rapeseed, Wine and the french Paradoxon

Resveratrol – The Vision

Background:

Resveratrol is known as health improving compound in red wine. Antioxidative and anticancerogen effects are in discussion.

- **Synthesis of resveratrol in rapeseed**
  - Establishment of a new source, enabling the uptake of this highly effective compound also under current nutritional habits in developped countries.
- **Transgenic approach:**
  - Integration of the gene for stilbensynthase (to synthesize resveratrol) and blocking of competing physiological pathways
- **Classical breeding:** not possible

Resveratrol – The Status

	RESVERA-TROL	SINAPINE Line	SINAPINE transgen
Useful materials		NPZ, DSV, Uni GÖ	
Genes and constructs	NPZ		IPB
Transformation Mulitplication	**SU Lab NPZ, IPB**		**Uni Gö IPB,DSV**
Breeding	NPZ, DSV		
Processing	**IVV, Raiffeisen**		
Oil	BASF		
Lecithine		Deg. Text.	
Condensate			
Aqueous phase		IVV, Rovita	
Nutritional physiology	DFA, Uni KI FH MÜ, ITEM		
Soc.-econ. implications	Uni HH		

As of: April 2004

- Production of transgenic lines with 25 fold higher content of Resveratrol glucosides vs.red wine
  - Single transformants STS
    - T3-bulk: up to **258 mg/kg** seeds
  - Co transformants STS + antisense SGT
    - T3-bulk: up to **424 mg/kg** seeds

- Extraction trials (fractionating properties)
  - Presence nearly completely in the meal after oil extraction
  - Seed conditioning using steam under pressure: transfer into oil?

- Toxicology and physiological effects of *trans*-Resveratrol
  - Glucuronisation of Resveratrol in vivo
  - Toxicolog. critical results in vitro and at high dosages in vivo

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Subproject Protein/Sinapine

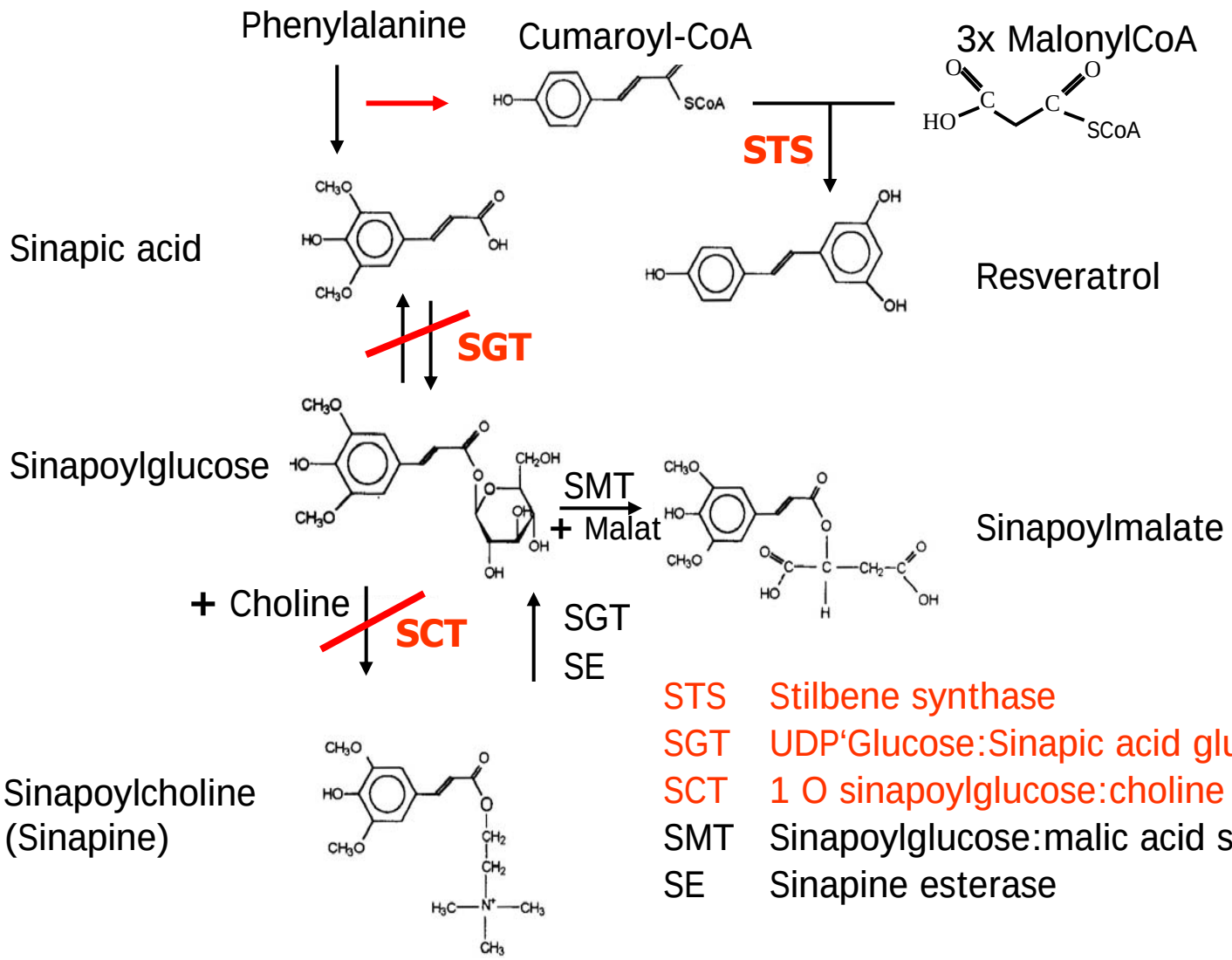
About Egg white, Bitter compounds and Yellow Seeds

Protein/Sinapine - The Vision

Background:
The biological value of rapeseed protein is very high due to a lot of essential amino acids in a valuable combination.

- Use of the high value rapeseed protein for human consumption
- Necessary actions:
  - Reduction of the phenolic compound Sinapine
  - Development of yellow seed rapeseed lines
  - Development of appropriate fractioning technics for simultanous extraction of protein and oil
  - Refunctionalizing of proteins for food use

Protein/Sinapine – The Strategy



STS	Stilbene synthase
SGT	UDP‘Glucose:Sinapic acid glucosyl transferase
SCT	1 O sinapoylglucose:choline sinapoyl transferase
SMT	Sinapoylglucose:malic acid sinapoyl transferas
SE	Sinapine esterase

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Protein/Sinapine – The Strategy

- **Transgenic breeding**
  - Reducing sinapine content by blocking enzymes of the sinapine metabolism
- **Classical breeding**
  - Selection of low sinapine canola lines
  - Selection of yellow seed canola lines
- **Processing**
  - Development of extraction methods for securing protein and oil quality
  - Technological optimization of protein quality
    - Refunctionalizing of proteins
  - Applications in foods

Protein – status quo

	SINAPINE Line	SINAPINE transgenic	Yellow-seed transgenic
Useful materials	NPZ, DSV, Uni GÖ		NPZ DSV
Genes and constructs		**IPB**	
Transformation Mulitplication		**Uni Gö** DSV, **IPB**	
Breeding	**NPZ, DSV, Uni GÖ**		
Processing	**IVV, Raiffeisen**		
Oil			
Lecithine	Deg. Text.		
Condensate			
Aqueous phase	**IVV, Rovita**		
Nutritional physiology			
Soc.-econ. implications	Uni HH		

As of: Sept. 2004

- Generation of transgenic plants with low sinapoyl contents
  - Single transformants antisense SGT: about 2 mg/g seed
- Indications on the regulation of Sinapin-metabolism

- High variance in classic breeding material
  - Sinapine content (<5 bis >12mg Sinapoyl/g seed)
- Development of a calibration for NIRS-analysis (Sinapine content)
- Selection of yellow seed lines

- Production + testing of proteins in food
  - Use of canola protein concentrate (2%) for the production of *sausages*

Lecithin

About Emulsifiers and Classical Breeding

degussa.

Texturant Systems

Lecithine – The Vision

Background
Lecithine is of multiple use in the food sector, e.g. as emulsifier or for pharmaceutical applications

- **Implementation of canola lecithine (as an alternative to soy lecithine)**
- Necessary steps:
  - Reduction of the phenolic compound sinapine
  - Development of yellow seed canola lines
  - Overexpression of new polar lipids in rapeseed

Lecithine – The Strategy

- **Transgenic breeding**
  - Accumulation of phospho- und glucolipides by integration of different enzymes of the phospho- und glucolipid metabolism
  - Accumulation of LCPUFAs in polarlipid fraction

- **Classic breeding**
  - Selection of yellow seed canola lines
  - Selection of gluco- und phospholipid high rapeseed lines
  - Advanced breeding work to develop conventional varieties

Lecithine – Status Quo

	YELLOW SEED	POLARLIPIDE Glyco-lipid	POLARLIPIDE Phospho-lipid
Useful materials	**NPZ DSV**		
Genes and constructs		**Uni HH**	
Transformation Mulitplication		**SU Labor, DSV**	
Breeding	**NPZ, DSV, Uni GÖ**		
Processing	IVV, Raiffeisen		
Oil			
Lecithine	**Deg. Text., Numico**		
Condensate			
Aqueous phase	IVV		
Nutritional physiology			
Soc.-econ. implications	Uni HH		

As of: Sept. 2004

- Isolation and analysis of different types of genes for the over expression of glyco- and phospholipids and for membrane proliferation
- Development of transgenic plants

- Evidence of variation in classical material
  - Different types of polar lipid fractions
- Development of yellow seed lines

- Development of new analytical methods
- Realization of extraction- and application experiments
  - e.g. baking experiments

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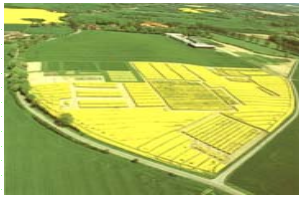
Napus 2000 – Functional food from transgenic Rapeseed



Cooperative project supported by the BMBF

- **Neutral Lipids / LCPUFAs: transgenic approach**
  - Production of the worldwide first transgenic LCPUFA-linseed plants: **2.0 % ARA, 1.5% EPA**
- **Tocopherols: transgenic and classical breeding**
  - Selection of high tocopherol oilseed rape, vitamin E content in oil: **1300 ppm total tocopherol**
- **Resveratrol: transgenic approach**
  - Breeding of transgenic lines with high content of resveratrol: **25 fold higher than in red wine**
- **Protein: transgenic and classical breeding**
  - Breeding of transgenic canola with low content of sinapine: **≤ 2 mg/g seed with transgenic approach**
    ≤ 5 mg/g seed with classical breeding

Total budget: 20.5 mio €, thereof 13.6 mio € BMBF funds over 5 years (Oct. 1999 – Nov. 2005)

NAPUS 2000

Acknowledgements

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- Norddeutsche Pflanzenzucht Hans Georg Lembke KG
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- Numico Research Group Germany
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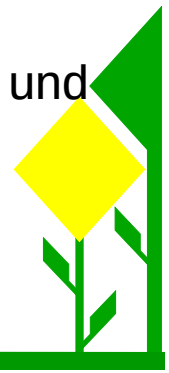

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